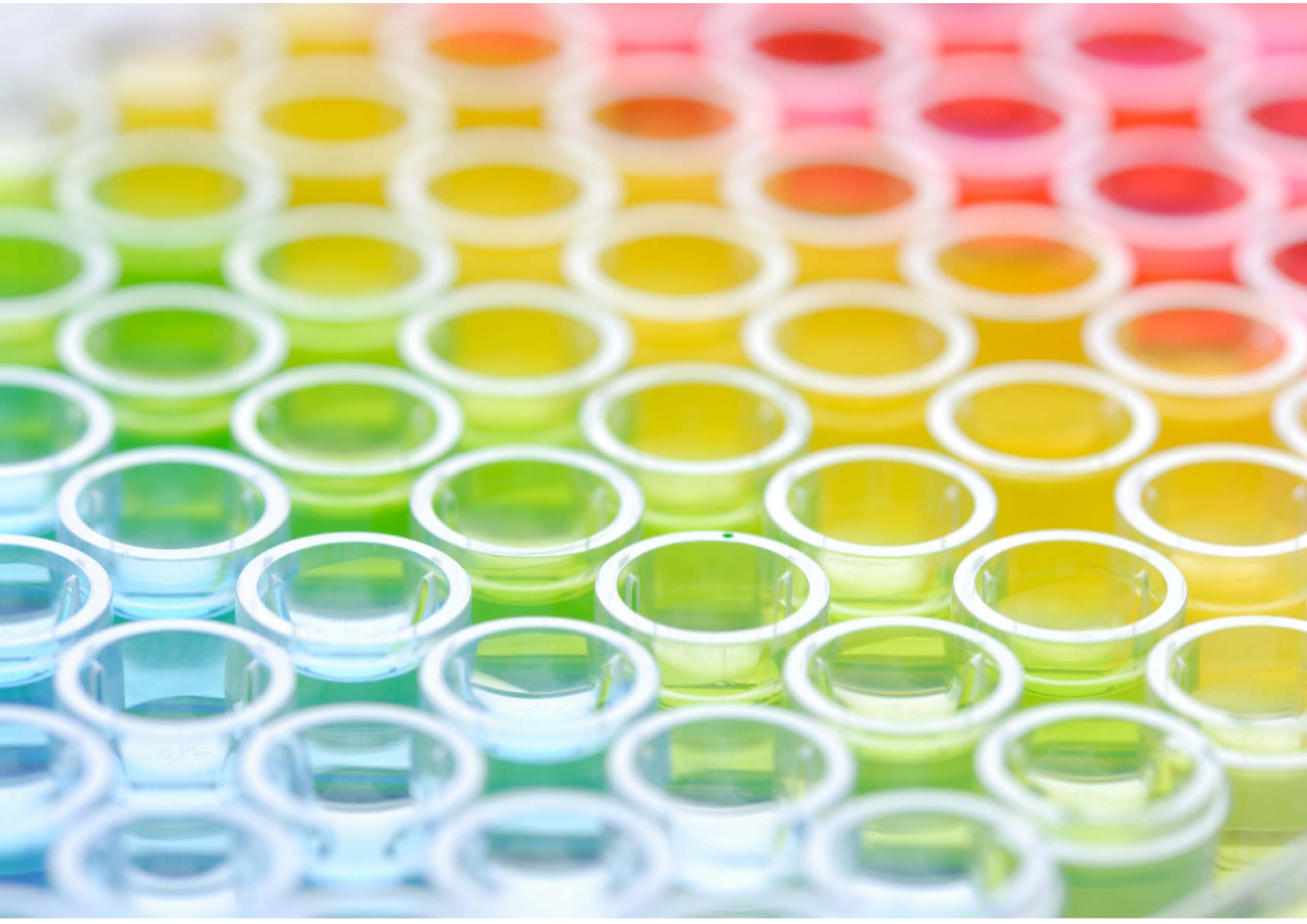
ImTec DIAGNOSTICS NV

Enzyme Probes & Assay Kits

2016-2017



Absorbance | **Fluorescence** | **Luminescence**

AAT Bioquest®

Advancing Assay & Test Technologies

Our Mission

AAT Bioquest® is committed to constantly meet or exceed its customer's requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

Our Story

AAT Bioquest®, Inc. (formerly ABD Bioquest, Inc.) develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest offers a rapidly expanding list of enabling products. Besides the standard catalog products, we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

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1. Prices, Orders and Changes: Prices shown are in US currency. Please call us for current prices if you require this information prior to placing your order. We guarantee our written quotations for 60 days. You may not cancel purchase orders unless such cancellation is expressly agreed by us. In such event, you will be advised of the total charge for such cancellation. You agree to pay such charges, including, but not limited to, storage and shipment costs, costs of producing non-standard materials, costs of purchasing non-returnable materials, cancellation costs imposed on us by our suppliers, and any other cost resulting from cancellation of this order.

2. Delivery: In most cases, we use standard overnight or two-day Federal Express delivery (or equivalent). All shipping charges billed are the responsibility of the customer and are normally prepaid by AAT Bioquest, Inc. and added to the invoice. We reserve the right to make delivery in installments, all such installments to be separately invoiced and paid for when due per invoice, without regard to subsequent deliveries. Partial shipments of available items are made when another item is backordered. Please inspect your packages upon receipt. If the goods have been damaged in transit, we can assist you in filing a claim with the carrier. You shall notify us in writing of any claims for shortages, defects or damages and shall hold the goods for our written instructions concerning disposition. Any claims for such errors must be made within 10 business days. If it is our error, we will do whatever is necessary to ship the correct products as soon as possible. If you shall fail to notify us any defects within 10 days after the goods have been received, such goods shall conclusively be deemed to conform to the terms and conditions and to have been irrevocably accepted by the buyer.

3. Payment: Terms of sale are net 30 days of date of invoice that is sent to you within 24 hours of shipping the order. The amount received must be sufficient to cover both the invoiced amount and any bank charges that may be incurred. Late charges may be added to invoices not paid within the 30-day time period. Late charges must be paid before subsequent orders can be shipped.

4. Warranties: The products shipped by AAT Bioquest are warranted to conform to the chemical or biological descriptions provided in our publications. This warranty is exclusive, and we makes no other warranty, express or implied, including any implied warranty of merchantability or fitness for any particular purpose. Our sole and exclusive liability and your exclusive remedy with respect to products proved to our satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, in our sole discretion, upon the return of such products in accordance with our instructions. We will not be liable for any incidental, consequential or contingent damages involving their use.

5. Returns: We must authorize any returns. We will not accept return shipments unless we have given prior written permission and shipping instructions. Goods may not be returned for credit except with our permission, and then only in strict compliance with our return shipment instructions. Any returned items may be subject to a 20% restocking fee. In many cases, items ordered in error cannot be returned because of the sensitive nature of many of our products and the difficulty and expense of requalifying returned items. If items are accepted for return, they must be in new, unopened, unused and undamaged condition, and you will be charged a per-unit 20% restocking charge.

6. Use of Our Products: Our products are used ONLY for laboratory research and development purposes. We realize that, since our products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. You assume responsibility to assure that the products purchased from us are approved for use under TSCA, if applicable. You have the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from us. You also have the duty to warn your customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products.

7. Patent Disclaimer: We do not warrant that the use or sale of our products will not infringe the claims of any United States or other patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

8. Miscellaneous: We reserve the right to discontinue our products or change specifications or prices of our products and to correct any errors or omissions at any time without incurring obligations.

Custom Products and Services

Our Technologies

Amplite™ enzyme-based detection platform is optimized for measuring horseradish peroxidase (HRP), alkaline phosphates, luciferase, beta-galactosidase, lactamase, oxidase, protein kinases, protein phosphatases, phosphodiesterases, proteases, cytochrome P450, histone deacetylase (HDAC) and cell signaling molecules such as NAD/NADH, NADP/NADPH, IP_3, cAMP and cGMP etc.

Cell Explorer™ cell labeling platform is a complete set of tools for tracking live cells. This platform is also widely used for sorting mixed populations of cells.

Cell Navigator™ cell staining platform is a complete set of tools for selective labeling subcellular structures of live, fixed and dead cells.

Cell Meter™ cellular functional assay platform is a complete set of tools for functional analysis of cellular events and real time-monitoring of cell functions.

iFluor™ superior fluorescent labeling dyes are optimized for labeling proteins and nucleic acids. This group of dyes span from UV to infrared wavelength with good photostability and brightness.

mFluor™ superior fluorescent labeling dyes are optimized for flow cytometry applications.

PhosphoWorks™ detection platform is a set of tools for detection of ATP, ADP, AMP, phosphate, pyrophosphate, phosphoproteins and phosphopeptides.

Quest View™ colorimetric protease platform is a sensitive and robust tool for rapid detection of protease and glycosidase biomarkers. This technology platform has been licensed by a few diagnostic companies for developing rapid diagnostic tests.

RatioWorks™ superior cellular dyes are a sensitive and robust tool set for ratio imaging and real time monitoring of cellular functions (such as pH and ions) in live cells.

Screen Quest™ assay kits are a set of HTS-ready tools for high throughput screening of biochemical and cellular targets such as protein kinases, proteases, HDAC, cell apoptosis and cytotoxicity, GPCR, ion channels, ADME and transporters.

Tide Fluor™ and Tide Quencher™ superior labeling dyes are specially optimized for labeling nucleotides and peptides. This platform offers the best value in the industry. It is second to none in terms of performance and cost. This technology platform has been licensed by a few diagnostic companies for developing IVD diagnostic tests.

trFluor™ superior fluorescent labeling dyes are optimized for developing time-resolved fluorescence-based assays. It has been used for developing HTS assay technologies for many drug discovery targets.

Our Services

Besides the catalog products we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays, custom bioconjugation and custom high throughput screening of drug discovery targets.

Custom Assay Design and Development

At AAT Bioquest we not only make probes and assay kits, but also use them extensively ourselves. Scientists at AAT Bioquest are experts on assay design and have developed a wide variety of tests that range from biochemical detection to cellular functions. Our assay options include:

- Enzyme activities
- Binding assays
- Cell-based assays
- Microplate assays
- Flow cytometric analysis
- Fluorescence imaging

Custom Conjugation

AAT Bioquest offers the best and the most rapid bioconjugation service in the industry.

- Biotinylation
- Fluorescence labeling (iFluor™, mFluor™, APC, RPE and PerCP)
- Enzyme labeling (AP and HRP)
- Small molecule conjugation

Custom Screening

AAT Bioquest offers on-demand high-throughput screening and pharmacology profiling assays with multiple methodologies. Functional assays are designed, validated and customized to the needs of our pharmaceutical and biotechnology industry clients. These assays are aimed at assessing and monitoring the efficacy, tolerability and safety parameters of candidate compounds for treating and/or diagnosing cancer, infectious disease, autoimmunity and transplantation. Our screening options include:

- Full assay development for a target of your choice
- Optimization of your assay protocol for HTS
- Multiple assay platforms and detection methods
- Custom data analysis

Custom Synthesis of Fluorophores and Luminophores

AAT Bioquest is recognized by the top pharmaceutical companies and diagnostic companies as a key provider of novel fluorescent dyes and luminescent probes. Over the years we have developed and synthesized many enabling fluorescent and luminescent probes for running a variety of challenging biological detection tasks.

Introduction 2

section contents at-a-glance

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Introduction to Fluorescence-Based Assays

2.1 Fluorescence-Based Assays

Fluorescence is the molecular absorption of light energy at one wavelength and its nearly instantaneous emission at another wavelength (usually longer). Light is absorbed by molecules in about 10^{-15} seconds which causes electrons to become excited to a higher electronic state. The electrons remain in the excited state for about 10^{-8} seconds, and then return to the ground state assuming all of the excess energy is not lost by collisions with other molecules. Energy is emitted during the time when electrons return to their ground state. Emitted light always has a longer wavelength than the absorbed light due to limited energy loss by the molecule prior to emission. This process is illustrated in Figure 2.1.

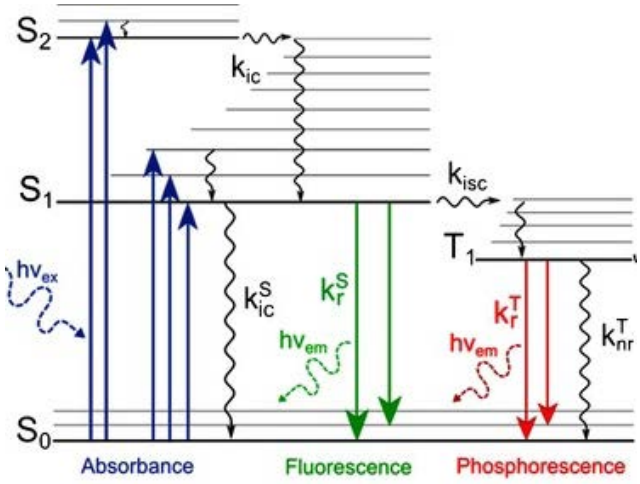

Figure 2.1. The Jablonski diagram illustrating the creation of fluorescence

Fluorescent compounds have two characteristic spectra: an excitation spectrum (the wavelength and amount of light absorbed) and an emission spectrum (the wavelength and amount of light emitted). Both absorption and radiation (emission) of energy are unique characteristics of a particular molecule during the fluorescence process. Fluorescence measurements are chosen for their extraordinary sensitivity, high specificity, simplicity, and low cost as compared to other analytical modes. Fluorescence measurements can be 10-1000 folds more sensitive than absorbance measurements. They are widely accepted and powerful techniques that are used for a variety of environmental, industrial, and biotechnology applications. They are valuable analytical tools for both quantitative and qualitative analysis.

- ***High Sensitivity:*** Limits of detection largely depend on the properties of the molecule and surrounding environments being measured. ppb (parts per billion) or even ppt (parts per trillion) detection limit is achievable for most analytes. This extraordinary sensitivity allows the reliable detection of fluorescent materials even using small sample sizes.

- ***Low Interference:*** Spectrophotometric measurement of light absorption by an analyte is prone to interference problems because many materials absorb light, making it difficult to isolate the targeted analyte in a complex matrix. Fluorimetric measurements are highly specific and less susceptible to interferences because much fewer materials absorb and also emit light (fluorescence).

- ***Large Dynamic Range:*** Fluorescence output is linear to sample concentration over a very broad range. Fluorimetric measurements can be used over three to six magnitudes of concentration without sample dilution or modification of the sample cell.

- ***High Throughput:*** Fluorimetric measurement is a relatively simple analytical technique. Its high sensitivity and low interference reduce or eliminate the sample preparation procedures that often require to concentrate analytes or to remove interferences from samples prior to analysis. Most fluorescence-based assays can be automated for high throughput screening applications.

2.2 Fluorescence Instruments

There are three primary kinds of instruments that measure fluorescence: spectrofluorometers (e.g. fluorometers, flow cytometers and microplate readers), fluorescence scanners (e.g. equipment for electrophoresis and microarrays) and fluorescence imagers (e.g. microscopes). A generic fluorescence detection system consists of the following essential components:

- ***Light Source:*** The light source provides the energy that excites the compound of interest by emitting light. Light sources include xenon lamps, high pressure mercury vapor lamps, xenon-mercury arc lamps, lasers and LEDs. Lamps emit a broad range of light that has more wavelengths than those required to excite the compound. Xenon lamps are very versatile and powerful, providing light output from 190-1200 nm. Mercury vapor lamps are usually more intense than xenon lamps, but the intensity is concentrated in wavelengths of the Hg spectrum. Convenient and inexpensive tunable lasers have long been sought for spectroscopic uses. Lasers and LEDs emit more specific wavelengths. Most fluorescence instruments are equipped with 488 nm excitation of Argon laser.

- ***Excitation Filter:*** The excitation filter is used to screen out the wavelengths of light not absorbed by the compound being measured. This filter allows a selected band of light energy to pass through and excite the sample. It blocks other wavelengths, especially those in the emission spectrum.

- ***Optical Filters:*** Although more monochromator-based scanning fluorescence instruments are becoming available, there are many fluorescence instruments that still require filters. Optical filters are chosen to be optimal for each application, cost effective, and durable. Filters are used to selectively pass a portion of the ultraviolet or visible spectrum. In combination with a light source, the excitation filter allows only light which excites the molecule of interest to strike the sample. The emission filter allows the fluorescence from the sample to pass to the detector and blocks stray light from the light source or interfering components in the sample.

- ***Photodetector:*** The detection limit of a fluorescence instrument largely depends upon the detector that it uses. There are three major classes of photodetectors: photoemissive devices (e.g. photomultiplier tube), charge-coupled devices (CCD) and photoconductive devices (e.g. light-dependent resistor).

There is a large number of innovative fluorescence instruments developed for biological applications. To choose an appropriate fluorescence instrument for your research, there are a few critical factors. Sensitivity, dynamic range, stability and throughput are important instrument factors to be considered.

- ***Sensitivity:*** Sensitivity of a fluorometer refers to the minimum detectable quantity of a compound of interest under specified instrument conditions. It is related to two factors: signal-to-noise and signal-to-blank. Practically, sensitivity means the minimum concentration that can be measured above background fluorescence of the interferences. Note that when comparing two instruments for sensitivity, absolute numbers are meaningless. One cannot simply read a sample and a blank in two instruments and say the instrument with the "higher" numbers is more sensitive.

- ***Signal-to-Noise:*** Signal refers to the reading of light passed through a sample. Noise refers to the output from the instrument's electronics, which is present whether or not sample is being read.

- ***Signal-to-Blank:*** This is related to signal-to-noise but not the same. Signal refers to the reading of a sample. Blank refers to the matrix liquid containing none of the compound to be measured and scattered light. Signal-to-blank ratio can be determined by measuring blank against sample concentration and determining the ratio. Signal-to-blank ratio can be improved by employing better optics for the specific chemistry. A comparison of minimum detection limits among fluorometers is often made by using a stable fluorescent compound as a reference standard. This can work well in many cases, provided the instruments are properly and "equivalently" set up and operated. The standard must be pure and properly diluted and stable. AAT Bioquest offers a number of fluorescence reference standard compounds for fluorescence instrument calibration. These products are carefully chosen and purified, and we guarantee that these compounds give the same corrected fluorescence spectra from batch to batch. Our fluorescence instrument calibration kits contain a set of stable and water-soluble dyes to cover the full fluorescence spectrum.

- ***Dynamic Range:*** Dynamic range refers to the range of concentrations an instrument can read, from the minimum to the maximum detectable. The minimum detectable concentration is determined by signal-to-noise and signal-to-blank ratios. The maximum detectable concentration is determined by the compound's chemistry and by factors such as instrument sensitivity ranges, optical path length, specificity of optical filters, etc.

- ***Instrument Stability:*** An electronically stable fluorescence instrument is especially important to produce consistent analytical results over long periods of time.

- ***Instrument Throughput:*** The throughput of a fluorescence instrument becomes increasingly important. High throughput screening of drug molecules has become an essential part in drug discovery. There are many advanced fluorescence detection systems dedicated for drug discovery applications and other biological applications, e.g. IN Cell Analysis Systems and LEADseeker (GE Healthcare), FLIPR™ microplate reader (Molecular Devices Corporation), FDSS 7000EX and FDSS µCELL (Hamamatsu), ArrayScan® HCS Reader (ThermoFisher.) and ClonePix System (Molecular Devices).

2.3 Selection of Fluorescent Reagents for Enzyme Assays

Fluorescence is a technology that is now used routinely in life science research. Fluorescence reagents are used extensively to trace the presence of biomolecules in cells and other biological systems. The great advance of fluorescence reagents has promoted a host of more complex fluorescence technologies such as fluorescence resonance energy transfer (FRET), time-resolved fluorescence (TRF), fluorescence polarization (FP), fluorescence recovery after photobleaching (FRAP), fluorescence activated cell sorting (FACS) and fluorescence correlation spectroscopy (FCS) etc. Excitation and emission wavelength, fluorescence quantum yield, fluorescence lifetime, size, photostability and biological functionality are important factors to be considered in selection of a desired fluorescent probe for your applications. Besides the fluorescence instrument discussed above, fluorescent reagents are the most critical factor in the successful use of fluorescence technologies. There are several factors that need be considered in the selection of appropriate fluorescent reagents for your assays.

- ***Measurement Modality:*** Fluorescent dyes are used to track biological events through their fluorescence changes that respond to the biological events of interest. Fluorescence changes are measured in three essential modes: fluorescence intensity, fluorescence lifetime and fluorescence polarization. Fluorescence intensity measurement is still the predominant mode although measurements of fluorescence polarization and fluorescence lifetime get more attentions in recent years.

- ***Excitation and Emission Wavelength:*** There are many factors to be considered in selection of appropriate excitation and emission wavelengths, e.g. the light source and filters of the fluorescence instrument used, the absorption and emission of undesired impurities in the analyte. In general, longer wavelengths tend to give better sensitivity.

- ***Band Shape and Width:*** The shape of the excitation and emission spectra is an important component in multiplexing applications. For organic dyes both the excitation and emission spectra usually have multiple peaks or shoulders as well as gradually diminishing tails to the red of the last peak. Inorganic materials (such as lanthanide complexes and quantum dots) display extremely symmetrical and narrow spectra that are very useful for multiplexing applications.

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- ***Stokes Shift:*** The Stokes shift is the difference between the absorption peak and the emission peak for fluorophores. Larger Stokes shift is always preferred as long as other properties of fluorescent probes are not compromised. Larger Stokes shift allows the use of broad excitation and emission filters that do not overlap, which increases brightness and sensitivity. Fluorescent probes of smaller Stokes shift requires filters that are very close together and do not include the entire area of the curves, thus reducing efficiency and brightness.

- ***Photostability:*** Many chemical processes lead to the degradation of the emission from conventional dyes. Photooxidation is the primary cause of photobleaching. There are two ways to reduce photobleaching: selecting more photostable fluorescent reagents or adding anti-oxidants (in the assay systems). For example, rhodamines are preferred over fluoresceins for photostability reason. In general, microscopic assays require more stringent photostability than microplate or flow cytometry-based assays.

As discussed above, there are many factors that have significant effects on both fluorescence instruments and fluorescent reagents. Besides these instrumentation and reagent effects, there are quite a few assay conditions that need be carefully controlled to give the best assay results. In general the following critical factors should be considered to develop a robust fluorescence-based assay. These factors include pH effect, environment effect, ion effect, enzyme action and receptor binding.

- ***Linearity and Dynamic Range:*** Fluorescence intensity is theoretically proportional (linear) to concentration. There are, however, factors that affect this linear relationship. When concentration is too high, light cannot pass through the sample to cause excitation. Thus very high concentrations can have very low fluorescence intensity (concentration quenching). The linearity of a sample is related to many factors, including the chemical composition of the sample and the path length the light must travel. An unknown sample should always be tested for linearity.

- ***Fluorescence Quenching:*** The term "quenching" refers to many factors that reduce or quench fluorescence. Quenching factors are one reason why it is very important to treat standards, blanks and samples in exactly the same manner.

- ***Solution Turbidity:*** Fluorescence measurements are significantly more immune to the effects of turbidity compared to absorption techniques like UV-VIS spectrophotometers. If the interfering substance is reflective, turbidity can create light scattering and readings will increase. If the interfering substance absorbs light, fluorescence will be reduced. If the interfering substance does not absorb light, however, the fluorescence readings will not be affected unless there is so much turbidity that the emitted light cannot penetrate the solvent.

- ***pH Effect:*** Fluorescence of many compounds is pH-sensitive. We recommend that buffers should be always used in your assays. In certain studies, pH factors can be an advantage. The pH dependence of probe molecules has been greatly used to determine the pH of cells and other biological systems.

- ***Photobleaching:*** Many fluorescent molecules can be bleached or destroyed by light. Ultraviolet light, especially, can cause certain molecules to break down. Fluorescence readings decrease as the molecules are destroyed. Rate of destruction varies depending upon environmental factors, including temperature.

- ***Temperature:*** Fluorescence is affected by changes in temperature. As temperature increases, fluorescence decreases. This might be due to an increase of molecular motion with increasing temperature, which results in more molecular collisions and subsequent loss of energy. However, for most fluorescent compounds the magnitude of temperature effect is much smaller than other effects described above.

2.4 Selection of Fluorescence Reference Standards for Instrument Calibration and Enzyme Detection

Fluorescence is a relative measurement and the optics and electronics of each instrument vary from manufacturer to manufacturer, even among instruments from the same manufacturer. A fluorescence instrument must be calibrated and recalibrated whenever the optics or filters are changed. As discussed above, fluorescence is subject to temperature and other environmental effects. It is important to calibrate the fluorometer in conditions as close as possible to the actual conditions for your study. Sample readings are only as accurate as the standard and blank used to calibrate the instrument. It is important to be rigorous in laboratory procedures, such as cleaning labware and carefully preparing standards. Most fluorescence instruments can be calibrated with well-characterized stable fluorescent dyes. We offer a number of fluorescence reference standard compounds for fluorescence instrument calibration. These products are carefully chosen and purified, and we guarantee that these compounds give the same corrected fluorescence spectra from batch to batch.

Assays designed to quantitate enzymatic activity typically employ substrates that yield highly fluorescent or intensely absorbing water-soluble products. An ideal fluorogenic substrate for fluorescence-based solution assays yields a highly fluorescent, water-soluble product with optical properties significantly different from those of the substrate. If the fluorescence spectra of the substrate and product overlap significantly, analysis will likely require a separation step, especially when using excess substrate to obtain pseudo–first-order kinetics. Fortunately, many substrates have low intrinsic fluorescence or are metabolized to products that have longer-wavelength excitation or emission spectra (Figure 2.2). These fluorescent products can typically be quantitated in the presence of the unreacted substrate using a fluorometer or a fluorescence microplate reader. Microplate readers facilitate high-throughput analysis and require relatively small assay volumes, which usually reduces reagent costs. Moreover, the front-face optics in many microplate readers allows researchers to use more concentrated solutions, which may both improve the linearity of the kinetics and

reduce inner-filter effects.

AMC (Cat# 51), AFC (Cat# 56) and Rhodamine 110 (Cat# 86) are used for calibrating the protease-induced enzymatic reactions that generate these fluorescent dyes from the corresponding fluorogenic enzyme substrates. Similarly, coumarins (Cat# 40, 41, 42 and 82), fluorescein (Cat# 2), resorufin (Cat# 65) and DDAO (Cat# 62). are used for calibrating other hydrolytic enzyme reactions that produce these phenolic dyes upon reactions with their corresponding fluorogenic enzyme substrates. These hydrolases include phosphatases and glycosidases, etc. D-Aminoluciferin (Cat# 13415) is used for calibrating the protease assays that use D-Aminoluciferin-based luminogenic enzyme substrates.

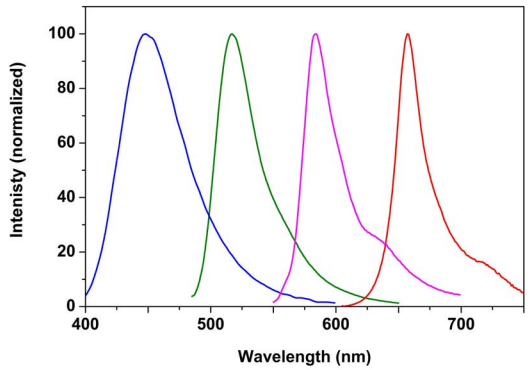

Figure 2.2. The normalized fluorescence spectra of 4-Methylumbelliferone (Cat# 41), fluorescein (Cat# 2), resorufin (Cat# 65) and DDAO (Cat# 62).

2.5 Fluorescence Resonance Energy Transfer (FRET)

FRET is a physical phenomenon, that is being used more and more in biomedical research and drug discovery today. FRET relies on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. Due to its sensitivity to distance, FRET has been used to investigate molecular interactions. FRET is the radiationless transmission of energy from a donor molecule to an acceptor molecule. The donor molecule is the dye or chromophore that initially absorbs the energy and the acceptor is the chromophore to which the energy is subsequently transferred. This resonance interaction occurs over greater than interatomic distances, without conversion to thermal energy and without any molecular collision. The transfer of energy leads to a reduction in the donor's fluorescence intensity and excited state lifetime, and an increase in the acceptor's emission intensity. A pair of molecules that interact in such a manner that FRET occurs is often referred to as a donor/acceptor pair.

While there are many factors that influence FRET, the primary conditions that need to be met in order for FRET to occur are relatively few. The donor and acceptor molecules must be in close proximity to one another (typically 10-100 Å). The absorption or excitation spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor. The degree to which they overlap is referred to as the spectral overlap integral (J). The donor and acceptor transition dipole orientations must be approximately parallel. Assuming that the donor acceptor pairs are compatible, the most critical element necessary for FRET to occur is close proximity of the pairs. Förster, demonstrated that the efficiency of the process (E) depends on the inverse sixth-distance between donor and acceptor as expressed with the following equation:

$$E = R_o^6/(R_o^6 + r^6)$$

Where R_o is the Förster distance at which half the energy is transferred and r is the actual distance between donor and acceptor. The distance at which energy transfer is 50% efficient is referred to as the Förster radius (R_o). The magnitude of the R_o is dependent on the spectral properties of the donor and the acceptor. Förster distances ranging from 20 to 90 Å are most useful for studies of biological macromolecules.

Table 2.1 Fluorescence Reference Standards for Enzyme Assays

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
56	AFC [7-Amino-4-trifluoromethylcoumarin] *Fluorescence Reference Standard*	1 g	380	500
51	AMC [7-Amino-4-methylcoumarin] *Fluorescence Reference Standard*	1 g	351	430
82	β-Trifluoromethylumbelliferone [7-Hydroxy-4-trifluoromethylcoumarin)] *Fluorescence Reference Standard*	100 mg	385	502
42	CF-MU [6-Chloro-8-fluoro-umbelliferone] *Fluorescence Reference Standard*	100 mg	362	452
40	3-Cyano-7-hydroxycoumarin *Fluorescence Reference Standard*	25 mg	408	450
13415	D-Aminoluciferin	10 mg	N/A	560
62	DDAO [7-Hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one)] *Fluorescence Reference Standard*	25 mg	646	659
2	Fluorescein, Disodium Salt *Fluorescence reference standard*	100 mg	490	514
41	7-Hydroxy-4-methylcoumarin [4-Methylumbelliferone] *Fluorescence Reference Standard*	1 g	360	450
65	Resorufin, Sodium Salt *Fluorescence Reference Standard*	100 mg	571	585
86	Rhodamine 110 *Fluorescence Reference Standard*	1 g	498	520

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Proteases 3

protease probes and assay kits at-a-glance*

Protease	Colorimetric	Fluorimetric			
		Blue	Green	Orange	Red
Generic Protease			13440	13441	
Caspase	13405, 13413, 13422	13401, 13402, 13403, 13410, 13411, 13420, 13421, 13425, 13426, 13502, 22795, 22812, 22813, 22820	13427, 13430, 13431, 13503, 20100, 20108, 20111, 20113, 20115, 20117, 20119, 20125, 22796, 22798, 22799, 22820, 22821, 22823		13433, 13434, 13435, 13504, 20101, 22797, 22816, 22817, 22820, 22822, 22850
MMP			13510, 13512, 13520, 13528		13511, 13521
Proteasome 20S		13453	13451, 13455, 13456, 13465		
Renin					13530

* Products listed by catalog number

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Proteases

3.1 Generic Protease Activity Assays

A protease is an enzyme that conducts proteolysis, i.e., the protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Proteases, also known as proteinases or proteolytic enzymes, are a large group of enzymes. They belong to the class of enzymes known as hydrolases, which catalyse the reaction of hydrolysis of various bonds with the participation of a water molecule. Proteases are divided into four major groups according to the character of their catalytic active site and conditions of action: serine proteinases, cysteine (thiol) proteinases, aspartic proteinases, and matrix metalloproteinases (MMPs). Attachment of a protease to a certain group depends on the structure of catalytic site and the amino acid (as one of the constituents) essential for its activity. Proteases are involved in digesting long protein chains into short fragments, splitting the peptide bonds that link amino acid residues. Some of them can detach the terminal amino acids from the protein chain (exopeptidases, such as aminopeptidases, carboxypeptidase A); the others attack internal peptide bonds of a protein (endopeptidases, such as trypsin, chymotrypsin, pepsin, papain and elastase).

Proteases occur in all organisms. These enzymes are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades (e.g., the blood-clotting cascade, the complement system, apoptosis pathways, and the invertebrate prophenoloxidase-activating cascade). Proteases can either break specific peptide bonds, depending on the amino acid sequence of a protein, or break down a complete peptide to amino acids. The activity can be a destructive change, abolishing a protein's function or digesting it to its principal components. It can be an activation of a function, or it can be a signal in a signaling pathway.

Proteases are used throughout an organism for various metabolic processes. Acid proteases secreted into the stomach (such as pepsin) and serine proteases present in duodenum (trypsin and chymotrypsin) enable us to digest the protein in food. Proteases present in blood serum (thrombin, plasmin, Hageman factor, etc.) play an important role in blood-clotting, as well as lysis of the clots, and the correct action of the immune system. Other proteases are present in leukocytes (elastase and cathepsin G) and play several different roles in metabolic control. Proteases determine the lifetime of other proteins playing an important physiological role like hormones, antibodies, or other enzymes—this is one of the fastest "switching on" and "switching off" regulatory mechanisms in the physiology of an organism. By complex cooperative action the proteases may proceed as cascade reactions, which result in rapid and efficient amplification of an organism's response to a physiological signal.

Bacteria also secrete proteases to hydrolyze (digest) the peptide bonds in proteins and therefore break the proteins down into their constituent monomers (amino acids). Bacterial and fungal proteases are particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated by nutritional signals in these organisms. The net impact of nutritional regulation of protease activity among the thousands of species present in soil can be observed at the overall microbial community level as proteins are broken down in response to carbon, nitrogen, or sulfur limitation. A secreted bacterial protease may also act as an exotoxin, and be an example of a virulence factor in bacterial pathogenesis. Bacterial exotoxic proteases destroy extracellular structures. Protease enzymes are also used extensively in the bread industry for bread improver. Protease assays are widely used for the investigation of protease inhibitors and the detection of protease activities. Monitoring various protease activities has become a routine task for many biological laboratories. Some proteases have been identified as good drug development targets.

Amplite™ Universal Fluorimetric Protease Activity Assay Kit (Cat# 13500) is an ideal choice to perform routine assays for the isolation of proteases, or for identifying the presence of contaminating proteases in protein samples. The kit uses a fluorescent casein conjugate which is proven to be a generic substrate for a broad spectrum of proteases (e.g. trypsin, chymotrypsin, thermolysin, proteinase K, protease XIV, and elastase). In the intact substrate, casein is heavily labeled with a green fluorescent dye, resulting in significant fluorescence quenching. Protease-catalyzed hydrolysis relieves its quenching effect, yielding brightly fluorescent dye-labeled short peptides. The increase in fluorescence intensity is directly proportional to protease activity. The assay can be performed in a convenient 96-well or 384-well microtiter plate format and readily adapted to automation. Its signal can be easily read with a fluorescence microplate reader at Ex/Em = 490/525 nm using the FITC filter set.

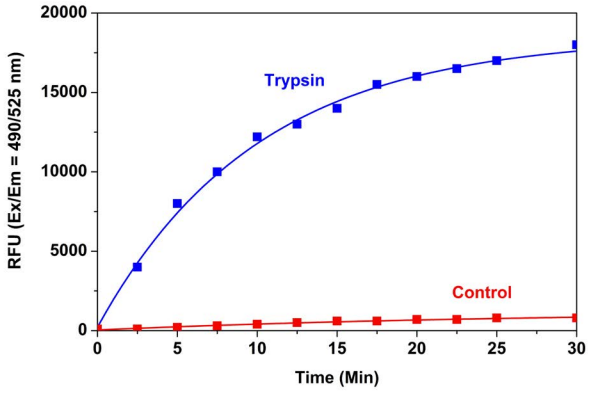

Figure 3.1. Trypsin protease activity was analyzed by Amplite™ Universal Fluorimetric Protease Activity Assay Kit (Cat# 13500). Protease substrate was incubated with 1 unit trypsin in the kit assay buffer. The control wells had protease substrate only (without trypsin). The fluorescence signals were measured starting from time 0 when trypsin was added. Samples were done in triplicates.

Table 3.1 Generic Protease Activity Probes and Assay Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13500	Amplite™ Universal Fluorimetric Protease Activity Assay Kit	500 tests	494	521
13440	Casein, FITC-conjugated	5 mg	494	521
13441	Casein, TAMRA-conjugated	5 mg	545	576

3.2 Caspases

A distinctive feature of the early stages of apoptosis is the activation of caspase enzymes. Members of the caspase (CED-3/ICE) family of cysteine–aspartic acid specific proteases have been identified as crucial mediators of the complex biochemical events associated with apoptosis. The recognition site for caspases is marked by three to four amino acids followed by an aspartic acid residue, with the cleavage occurring after the aspartate. The caspase proteases are typically synthesized as inactive precursors. Inhibitor release or cofactor binding activates the caspases through cleavage at internal aspartates, either by autocatalysis or by the action of another protease.

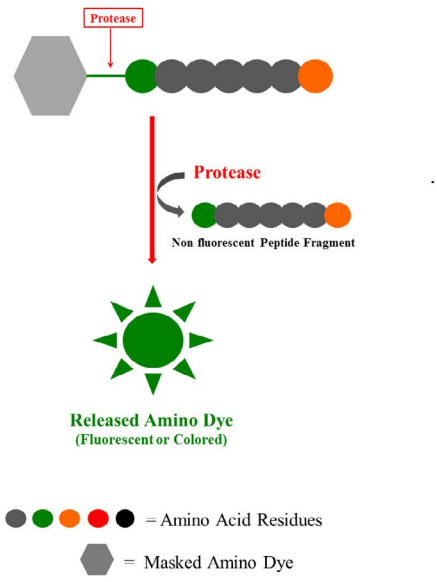

Figure 3.2. The caspase-sensitive peptide fragment-masked amino dyes are digested by a caspase to generate the highly fluorescent dye (or a highly colored dye). The fluorescence (or color) intensity increase is proportional to the caspase activity.

AAT Bioquest offers a diverse selection of capase inhibitors, chromogenic and fluorogenic caspase substrates, and caspase assay kits. Our chromogenic caspase substrates are based on 4-nitroaniline (*p*NA). AAT Bioquest is the only company that offers the multicolor substrates of four distinct fluorescence colors based on 7-Amino-4-methylcoumarin (AMC), 7-Amino-4-trifluoromethyl-coumarin (AFC), Rhodamine 110 (R110) and ProRed™ respectively (see Figure 3.3). In particular, the ProRed™-based caspase substrates are extremely useful for screening caspase inhibitors due to their longer excitation and emission wavelengths that eliminate the autofluorescence interference from the compound library.

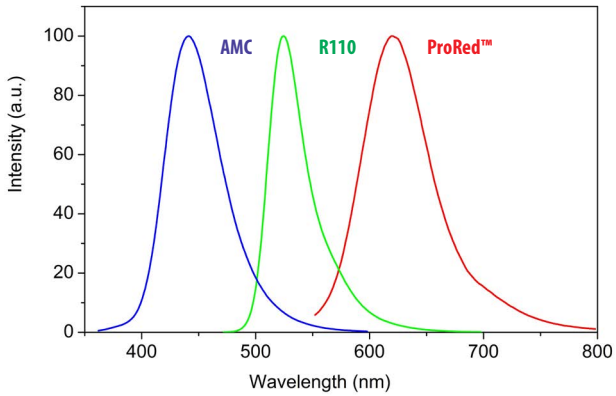

Figure 3.3. The fluoresence spectra of AMC (Cat# 51), R110 (Cat# 86) and ProRed™ in aqueous buffer (pH 7.0). AMC, R110 and ProRed™ caspase substrates are well suited for multiplexing caspase activities.

DEVD peptide sequence is selective for caspase 3/7. It has been used to develop a number of caspase 3/7 substrates. The Z-DEVD-R110 substrate is a nonfluorescent bisamide that is first converted by caspase 3/7 (or a closely related protease) to the fluorescent monoamide and then to the even more fluorescent R110 (excitation/emission maxima ~496/520 nm). R110-based caspase substrates are more sensitive than coumarin-based caspases substrates (e.g., AMC and AFC), but have narrower dynamic ranges due to the two-step cleavage process. We recommend that R110-based caspase substrates be used for end point assays while AMC and AFC

Table 3.2 Features of Different Apoptosis Probes

Parameters Measured	Probes	Key Features
Plasma Membrane Alterations (PS Exposure)	Annexin Binding Assay	Detect early apoptosis markers Flow cytometry or immunofluorescence application
Caspase Activation (Cytoplasm)	Caspasae Activity Assay	Quick, easy and high throughput
Caspase Binding (Cytoplasm)	Fluorescent Caspase Inhibitors	ELISA, flow cytometry, or Western blot
DNA Fragmentation (Nucleus)	BrdU Assay TUNEL Assay	Work with adherent cells, conjugated single cell resolution with cell cycle analysis by flow cytometry
Mitochondrial Changes	Mitochondrial Stains	Fast, easy, single cell resolution using flow cytometry or fluorescence microscopy

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caspase substrates are used for kinetic assays. Our ProRed™-DEVD substrates are extremely useful for screening caspase 3/7 inhibitors due to its longer excitation and emission wavelengths.

IETD petide sequence is selective for caspase 8. The Z-IETD-R110 substrate is a nonfluorescent bisamide that is first converted by caspase to the fluorescent monoamide and then to the even more fluorescent rhodamine 110 (excitation/emission maxima ~496/520 nm). R110-based caspase 8 substrate is more sensitive than coumarin-based caspases substrates (e.g., AMC and AFC), but has narrower dynamic range due to the two step cleavage process. We recommend that R110-based caspase 8 substrate be used for end point assays while AMC and AFC caspase substrates are used for kinetic assays. Our ProRed™-IETD substrate is extremely useful for screening caspase 8 inhibitors due to its longer excitation and emission wavelengths.

LEHD petide sequence is selective for caspase 9. DEVD and IETD-based peptides are selective for caspases 3/7 and 8 substrates respectively. We recommend that LEHD-R110-based caspase 9 substrate be used for end point assays while LEHD-AMC and LEHD-AFC caspase substrates be used for kinetic assays. Our ProRed™-LEHD substrate is extremely useful for screening caspase 9 inhibitors due to its longer excitation and emission wavelengths.

Caspase 3/7 Detection

Caspase 3 (CPP32/apopain) is a key effector in the apoptosis pathway, amplifying the signal from initiator caspases (such as caspase 8) and signifying full commitment to cellular disassembly. In addition to cleaving other caspases in the enzyme cascade, caspase 3 has been shown to cleave poly(ADP-ribose) polymerase (PARP), DNA-dependent protein kinase, protein kinase C_δ and actin.

Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit (Cat# 22797) is designed to monitor cell apoptosis through measuring caspase 3 activation. Caspase 3 is widely accepted as a reliable indicator for cell apoptosis since the activation of caspase 3 is important for the initiation of apoptosis. Caspase 3 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). Z-DEVD-ProRed™ 620 is used in the kit as the fluorogenic indicator for caspase 3 activity. Cleavage of Z-DEVD-ProRed™ 620 blocking peptide residue by caspase 3 generates strongly red fluorescent ProRed™ that is fluorimetrically monitored at ~620 nm with excitation at ~530 nm.

Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit (Cat# 22797) provides all the essential components with an optimized assay protocol. The assay is robust, and can be readily adapted for high-throughput assays in a wide variety of fluorescence platforms such as microplate assays. Using 100 µL of reagents per well in a 96-well format, the kit provides sufficient reagents to perform 100 assays. Using 25 µL of reagents per well in a 384-well format, the kit provides sufficient reagents to perform 400 assays.

Table 3.3 Caspase Activity Assay Reagents

Cat. #	Product Name	Biological Function	Size	Ex (nm)	Em (nm)
13401	Ac-DEVD-AFC	Fluorogenic Caspase 3/7 Substrate	5 mg	380	500
13402	Ac-DEVD-AMC	Fluorogenic Caspase 3/7 Substrate	5 mg	351	430
13403	Ac-DEVD-CHO	Caspase 3/7 Inhibitor	1 mg	N/A	N/A
13405	Ac-DEVD-pNA	Chromogenic Caspase 3/7 Substrate	5 mg	408	N/A
13410	Ac-IETD-AFC	Fluorogenic Caspase 8 Substrate	5 mg	380	500
13411	Ac-IETD-AMC	Fluorogenic Caspase 8 Substrate	5 mg	351	430
13412	Ac-IETD-CHO	Caspase 8 Inhibitor	5 mg	N/A	N/A
13431	$(Ac\text{-}IETD)_2$-R110	Fluorogenic Caspase 8 Substrate	1 mg	498	520
13426	Ac-LEHD-AMC	Fluorogenic Caspase 9 Substrate	5 mg	351	430
13427	$(Ac\text{-}LEHD)_2$-R110	Fluorogenic Caspase 9	1 mg	498	520
13420	Z-DEVD-AFC	Fluorogenic Caspase 3/7 Substrate	5 mg	380	500
13421	Z-DEVD-AMC	Fluorogenic Caspase 3/7 Substrate	5 mg	351	430
13422	Z-DEVD-pNA	Chromogenic Caspase 3/7 Substrate	5 mg	408	N/A
13433	Z-DEVD-ProRed™ 620	Fluorogenic Caspase 3/7 Substrate	1 mg	534	619
13430	$(Z\text{-}DEVD)_2$-R110	Fluorogenic Caspase 3/7 Substrate	1 mg	498	520
13425	Z-IETD-AFC	Fluorogenic Caspase 8 Substrate	5 mg	380	500
13413	Z-IETD-pNA	Chromogenic Caspase 8 Substrate	5 mg	408	N/A
13434	Z-IETD-ProRed™ 620	Fluorogenic Caspase 8 Substrate	1 mg	534	619
13435	Z-LEHD-ProRed™ 620	Fluorogenic Caspase 9 Substrate	1 mg	534	619

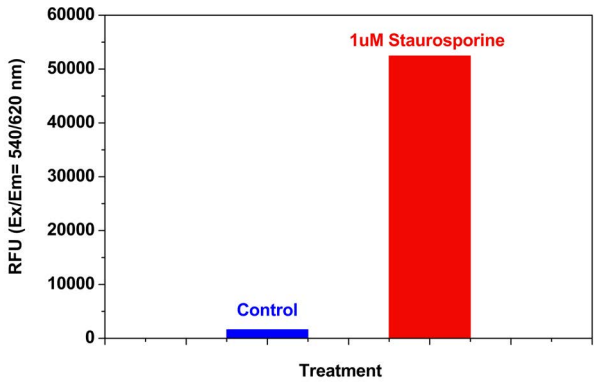

Figure 3.4. Detection of caspase 3/7 activities in Jurkat cells using Z-DEVD-ProRed™ 620 (Cat# 13433). Jurkat cells were seeded on the same day at 200,000 cells /well/90 µL in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 µM staurosporine for 5 hours. The caspase 3/7 assay solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 540/620 nm.

Table 3.4 Caspase 3/7 Activity Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13502	Amplite™ Fluorimetric Caspase 3/7 Assay Kit *Blue Fluorescence*	500 tests	351	430
13503	Amplite™ Fluorimetric Caspase 3/7 Assay Kit *Green Fluorescence*	500 tests	498	520
13504	Amplite™ Fluorimetric Caspase 3/7 Assay Kit *Red Fluorescence*	100 tests	534	619
22795	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 tests	351	430
22796	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Green Fluorescence*	200 tests	498	520
22797	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Red Fluorescence*	100 tests	534	619
22820	Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit *Triple Fluorescence*	3x100 tests	multi-color	multi-color

Caspase 8 Detection

Caspase 8 plays a critical role in the early cascade of apoptosis, acting as an initiator of the caspase activation cascade. Activation of the enzyme itself is accomplished through direct interaction with the death domains of cell-surface receptors for apoptosis-inducing ligands. The activated protease has been shown to be involved in a pathway that mediates the release of cytochrome c from the mitochondria, and is also known to activate downstream caspases, such as caspase 3. IETD peptide sequence is selective for caspase 8. AAT Bioquest offers both caspase reagents and assay kits for detecting caspase 8 (see Tables 3.3 and 3.5).

Table 3.5 Caspase 8 Activity Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22812	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 tests	351	430
22798	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Green Fluorescence*	200 tests	498	520
22816	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Red Fluorescence*	100 tests	534	619
22820	Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit *Triple Fluorescence*	100 tests	multi-color	multi-color

Caspase 9 Detection

Caspase 9 is a member of the CED-3 subfamily of the caspase family of cysteine proteases that play an essential role in the execution phase of apoptosis. LEHD peptide sequence is selective for caspase 9. AAT Bioquest offers pNA, AMC, AFC, R110 and ProRed™ caspase 9 substrates that contain the LEHD peptide fragment for caspase 9 selectivity (See Table 3.3 and 3.6).

Cell Meter™ Caspase 9 Activity Apoptosis Assay Kits are designed to monitor cell apoptosis by measuring caspase 9 activity. Kit #22799 uses $(Ac\text{-}LEHD)_2$-R110 as a fluorogenic indicator for caspase 9 activity while Kit #22813 uses Ac-LEHD-AMC to monitor caspase 9 activity. Cleavage of R110 peptides by caspase 9 generates strongly green fluorescent R110 while Ac-LEHD-AMC produces strongly blue fluorescent AMC upon interacting with caspase 9. Both kits provide all the essential components. The assays are robust and can be readily adapted for high throughput screening. They can be used to either quantify the activated caspase 9 activities in apoptotic cells or screen caspase 9 inhibitors.

Table 3.6 Caspase 9 Activity Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22813	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 tests	351	430
22799	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Green Fluorescence*	200 tests	498	520
22817	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Red Fluorescence*	100 tests	534	619
22820	Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit *Triple Fluorescence*	3x100 tests	multi-color	multi-color

Multiplexing Detection of Caspases 3/7, 8 and 9

AAT Bioquest has developed Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit (Cat# 22820) for multiplexing the detection of caspases 3/7, 8 and 9. This particular kit is designed to simultaneously monitor four key caspases (caspase 3/7, 8 and 9) activation involved in cell apoptosis using the three distinct fluorescent colors. This kit uses DEVD-ProRed™, IETD-R110 and LEHD-AMC as fluorogenic indicators for caspase 3/7, 8 and 9 activity respectively. Upon caspase cleavages, DEVD-ProRed™, IETD-

R110 and LEHD-AMC caspase substrates generate three distinct fluorophores: ProRed™ (red fluorescence), R110 (green fluorescence) and AMC (blue fluorescence), which can be readily monitored in a single assay due to their nice spectral separation (see Figure 3.3).

Table 3.7 Multiplexing Caspase Activity and Apoptosis Assay Kits

Cat. #	Product Name	Size
22840	Cell Meter™ Apoptotic and Necrotic Detection Kit *Triple Fluorescence*	100 tests
22843	Cell Meter™ Apoptotic and Necrotic Detection Kit *Triple Fluorescence*	100 tests
22820	Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit *Triple Fluorescence*	3x100 tests
22850	Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit *Triple Fluorescence*	100 tests

Caspase Binding Assays

In the process of apoptosis, one of the key events is the activation of caspases, which is important for the initiation of apoptosis. Cell Meter™ Live Cell Caspase Binding Kits use fluorescent cell permeable and nontoxic indicators to detect caspases 1, 2, 3/7, 6, 8, 9, 10, and 13 activities. Once bound to caspases, the fluorescent reagents are retained inside the cell. The binding event prevents the caspases from further catalysis but will not stop apoptosis from proceeding. The caspase binding kits are applicable for fluorescence microscope, flow cytometer, and fluorescence microplate reader. The kits provide all the essential components with an optimized assay protocol.

Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit (Cat# 22850) is designed to detect apoptosis by simultaneously monitoring Caspase 3/7 and Annexin V activities in mammalian cells. Annexins are a family of proteins that bind to phospholipid membranes in the presence of calcium. Annexin V is used to detect apoptotic cells that express phosphatidylserine (PS) on the cell surface. The appearance of PS on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis. Annexin V-dye conjugates monitor cell apoptosis through measuring the translocation of PS. The kit also provides a Hoechst dye for labeling the nucleus of the whole population of the cells, and propidium iodide dye for staining necrosis cells.

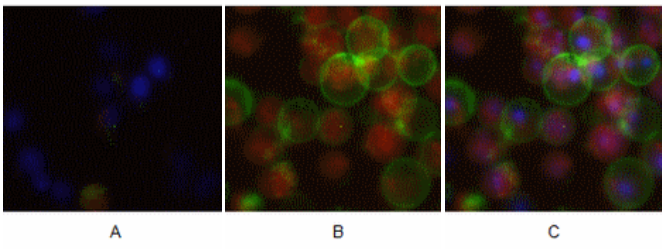

Figure 3.5. The fluorescence image analysis indicated the increased expression of caspase 3/7 (red, stained by TF3-DEVD-FMK) and Annexin V (green, stained by Annexin V-iFluor™ 488) in Jurkat cells induced by 1 µM staurosporine for 3 hours. The fluorescence images of the cells (300,000 cells/ well) were taken with Olympus fluorescence microscope using the DAPI, FITC, and TRITC channel respectively. Individual images of the same cell population were merged as shown above. A: Non-induced control cells; B: Double staining of staurosporine-induced cells for caspase 3/7 (red) and Annexin V (green); C. Triple staining of staurosporine-induced cells for caspase 3/7 (red), Annexin V (green) and nucleus (blue).

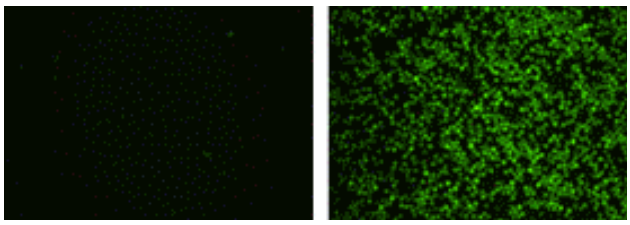

Figure 3.6. The fluorescence images demonstrated the increase in FITC-C6-DEVD-FMK (Cat# 13408) fluorescence intensity with the addition of 1 µM staurosporine in Jurkat cells. Cells were incubated with FITC-C6-DEVD-FMK for 1 hour at 37 ºC. The fluorescence intensity of the cells (200,000 cells/100 µL/well) was viewed under a fluorescence microscope using the FITC channel. A: Control; B: Staurosporine-treated .

Table 3.8 Caspase Binding Based Live Cell Apoptosis Reagents and Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
20108	Cell Meter™ Live Cell Caspase 1 Binding Assay Kit	25 tests	492	514
20111	Cell Meter™ Live Cell Caspase 2 Binding Assay Kit	25 tests	492	514
22850	Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit	100 tests	Multiple Color	
20100	Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit *Green Fluorescence*	25 tests	492	514
20101	Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit *Red Fluorescence*	25 tests	556	574
20113	Cell Meter™ Live Cell Caspase 6 Binding Assay Kit	25 tests	492	514
20115	Cell Meter™ Live Cell Caspase 8 Binding Assay Kit	25 tests	492	514
20117	Cell Meter™ Live Cell Caspase 9 Binding Assay Kit	25 tests	492	514
20119	Cell Meter™ Live Cell Caspase 10 Binding Assay Kit	25 tests	492	514
20125	Cell Meter™ Live Cell Caspase 13 Binding Assay Kit	25 tests	492	514
13470	FAM-VAD-FMK	25 tests	492	518
13408	FITC-C6-DEVD-FMK	100 µg	492	516
13409	FITC-C6-LEHD-FMK	100 µg	492	516
13475	mFluor™ 450-VAD-FMK	25 tests	403	454
13476	mFluor™ 510-VAD-FMK	25 tests	414	508
13472	SRB-VAD-FMK [Sulforhodamine B-VAD-FMK]	25 tests	556	575
13471	TF4-VAD-FMK	25 tests	588	610
13420	Z-DEVD-AFC	5 mg	380	500
13421	Z-DEVD-AMC	5 mg	351	430
13433	Z-DEVD-ProRed™ 620	1 mg	534	619
13435	Z-IEHD-ProRed™ 620	1 mg	534	619
13425	Z-IETD-AFC	5 mg	380	500
13434	Z-IETD-ProRed™ 620	1 mg	534	619

3.3 Matrix Metalloproteinases (MMP)

Amplite™ Universal Fluorimetric MMP Activity Assay Kit (Cat# 13510) uses a Tide Fluor™ 2 (TF2)/Tide Quencher™ 2 (TQ2) fluorescence resonance energy transfer (FRET) peptide as a generic MMP activity indicator. It is designed to check the general activity of a MMP enzyme and to screen MMP inhibitors. In the intact FRET peptide, the fluorescence of TF2 is quenched by TQ2. After cleaved into two separate fragments by MMPs, the fluorescence of TF2 is recovered. With excellent fluorescence quantum yield and longer wavelength, TF2 probe is much more sensitive than an EDANS/Dabcyl FRET substrate. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/525 nm.

Similar to Kit 13510, Kit 13511 uses a Tide Fluor™ 3 (TF3)/Tide Quencher™ 3 (TQ3) fluorescence resonance energy transfer (FRET) peptide as a MMP substrate. With excellent fluorescence quantum yield and longer wavelength, TF3 shows less interference from autofluorescence of test compounds and cellular components and is much more sensitive than an EDANS/Dabcyl FRET substrate. The pH-independent fluorescence of TF3 makes the assay reading available for the whole physiological pH range. The high photostability of TF3 might make the TF3/TQ3 FRET peptide a useful imaging probe. Many labs have used this kit for the high throughput screening of MMP inhibitors as potential anticancer drug candidates. This assay might be also used for monitoring cancer cells.

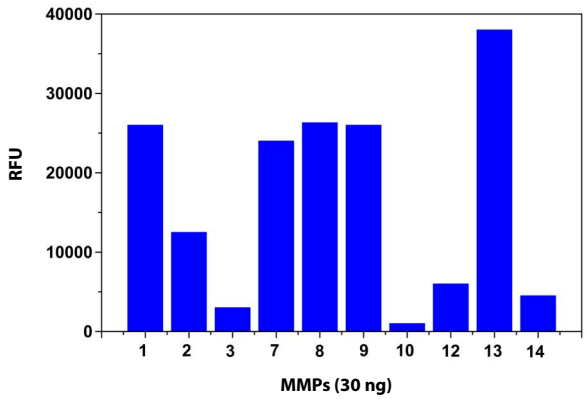

Figure 3.7. Detection of MMP activity using Amplite™ Universal Fluorimetric MMP Activity Assay Kit (Cat# 13510). The fluorescence signals were monitored one hour after the start of the reaction by using a NOVOStar microplate reader with a filter set of Ex/Em = 490/525 nm. The reading from all wells was subtracted with the reading from substrate control, which contained MMP Green™ substrate but no MMPs. The MMP Green™ substrate can detect the activity of sub-nanogram of all MMPs (n=3).

MMP-3 (Matrix Metalloproteinase-3), also known as stromelysin-1, constitutes a family of zinc-dependent endopeptidases that function within the extracellular matrix. These enzymes are responsible for the breakdown of connective tissues and are important in bone remodeling, the menstrual cycle, and repair of tissue damage. While the exact contribution of MMPs to certain pathological processes is difficult to assess, MMPs appear to play a key role in the development of arthritis as well as in the invasion and metastasis of cancer.

Amplite™ Fluorimetric MMP-3 Activity Assay Kit (Cat# 13512) is designed to monitor the activity of an MMP-3 enzyme. The poptide sequence used in the kit is more selective for MMP-3 hydrolysis than other MMP enzymes. It can also be used to screen MMP-3 inhibitors when a purified MMP-3 enzyme is used. The kit uses a Tide Fluor™ 2 (TF2)/Tide Quencher™ 2 (TQ2) fluorescence resonance energy transfer (FRET) peptide as the MMP-3 activity indicator. In the intact FRET peptide, the fluorescence of TF2 is quenched by TQ2. Upon cleavage into two separate fragments by MMP-3, the green fluorescence of TF2 is recovered. With excellent fluorescence quantum yield and longer wavelength, TF2 shows less interference from autofluorescence of test compounds and cellular components and is much more sensitive than an EDANS/Dabcyl FRET substrate. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. The perfect excitation of TF2 at 488 nm makes the assay readily readable with almost all the common fluorescence instruments equipped with Argon laser and FITC filter set.

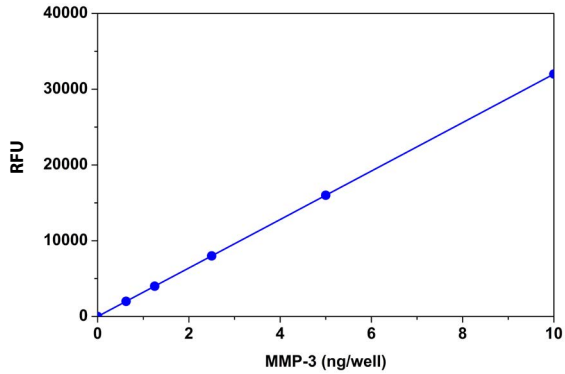

Figure 3.8. MMP-3 enzyme activity was measured with Kit 13512. As low as 1 ng/well MMP-3 was detected with 60 min incubation time (n=3). Note: MMP-3 from different sources varies in its endogenous activity.

Table 3.9 MMP Activity Assay Reagents and Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13512	Amplite™ Fluorimetric MMP-3 Activity Assay Kit *Green Fluorescence*	100 tests	498	520
13510	Amplite™ Universal Fluorimetric MMP Activity Assay Kit *Green Fluorescence*	100 tests	494	521
13511	Amplite™ Universal Fluorimetric MMP Activity Assay Kit *Red Fluorescence*	100 tests	545	576
13520	MMP Green™ Substrate	1 mg	498	520
13521	MMP Red™ Substrate	1 mg	545	576
13528	MMP-3 Green™ Substrate	1 mg	498	520

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3.4 Proteasome 20S

Proteasomes are protein complexes inside all eukaryotes and archaea, and in some bacteria. In eukaryotes, they are located in the nucleus and the cytoplasm. The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds. Enzymes that carry out such reactions are called proteases. Proteasomes are part of a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins. The degradation process yields peptides of about seven to eight amino acids long, which can then be further degraded into shorter amino acid sequences and used in synthesizing new proteins. Proteins are tagged for degradation with a small protein called ubiquitin. The tagging reaction is catalyzed by enzymes called ubiquitin ligases. Once a protein is tagged with a single ubiquitin molecule, this is a signal to other ligases to attach additional ubiquitin molecules. The result is a polyubiquitin chain that is bound by the proteasome, allowing it to degrade the tagged protein.

In structure, the proteasome is a cylindrical complex containing a "core" of four stacked rings forming a central pore. Each ring is composed of seven individual proteins. The inner two rings are made of seven β subunits that contain three to seven protease active sites. These sites are located on the interior surface of the rings, so that the target protein must enter the central pore before it is degraded. The outer two rings each contain seven α subunits whose function is to maintain a "gate" through which proteins enter the barrel. These α subunits are controlled by binding to "cap" structures or regulatory particles that recognize polyubiquitin tags attached to protein substrates and initiate the degradation process. The overall system of ubiquitination and proteasomal degradation is known as the ubiquitin-proteasome system.

The most common form of the proteasome in this pathway is the 26S proteasome, an ATP-dependent proteolytic complex, which contains one 20S (700-kDa) core particle structure and two 19S (700-kDa) regulatory caps. The 20S core contains three major proteolytic activities including chymotrypsin-like, trypsin-like and caspase-like activities. It is responsible for the breakdown of the key proteins involved with apoptosis, DNA repair, endocytosis, and cell cycle control.

Amplite™ Fluorimetric Proteasome 20S Activity Assay Kit (Cat# 13456) is a homogeneous fluorescent assay that measures the chymotrypsin-like protease activity associated with the proteasome complex in cultured cells. This kit uses LLVY-R110 as a fluorogenic indicator for proteasome activities. Cleavage of LLVY-R110 by proteasome generates strongly green fluorescent R110 that is monitored fluorimetrically at 520-530 nm with excitation at 480-500 nm. Fluorophore R110 has absorption maximum well suitable for 488 nm argon laser excitation. It has strong fluorescence from pH 2 to pH 10. The kit provides all the essential components with an optimized assay protocol. The assay is robust and can be readily adapted for high-throughput assays to evaluate the proteasome activities or screen the inhibitors in cultured cells or in solution. The kit has been used for screening biologically active compounds that interact with proteasome 20S.

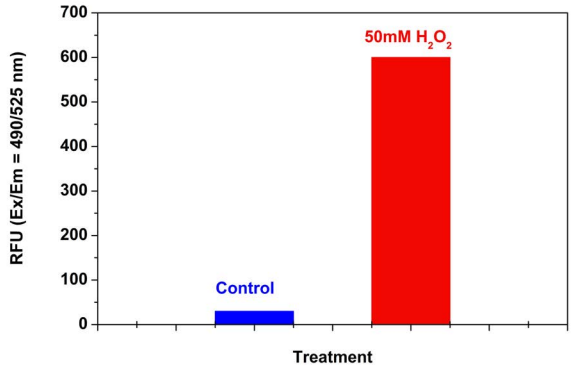

Figure 3.9. Detection of proteasome activity with Kit 13456 in Jurkat cells. Jurkat cells were seeded on the same day at 500,000 cells/90 µL/well in a 96-well black wall/clear bottom Costar plate. The cells were treated with or without 50 mM H_2O_2 for 30 minutes. The proteasome assay loading solution (100 µL/well) was added and incubated in a 5% CO_2, 37 °C incubator for 3 hours. The fluorescence intensity was measured at Ex/Em = 490/525 nm with Gemini fluorescent microplate reader (Molecular Devices).

Table 3.10 Proteasome 20S Detection Reagents and Assay Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	Function
13455	$(Ac\text{-}ANW)_2R110$	1 mg	498	520	Proteasome 20S-β5i
13465	$(Ac\text{-}KQL)_2R110$	1 mg	498	520	Proteasome 20S-β2i
13467	$(Ac\text{-}PAL)_2R110$	1 mg	498	520	Proteasome 20S-β1i
13468	$(Ac\text{-}WLA)_2R110$	1 mg	498	520	Proteasome 20S-β5c
13456	Amplite™ Fluorimetric Proteasome 20S Activity Assay Kit *Green Fluorescence*	1 plate	498	520	Proteasome 20S
13453	Suc-LLVY-AMC	1 mg	351	430	Proteasome 20S-β5c
13452	Suc-LLVY-Aminoluciferin	1 mg	N/A	560	Proteasome 20S-β5c
13451	$(Suc\text{-}LLVY)_2R110$	1 mg	498	520	Proteasome 20S-β5c
13466	$(Z\text{-}LLE)_2R110$	1 mg	498	520	Proteasome 20S-β1c

3.5 Renin

Renin is an enzyme that participates in the body's renin-angiotensin system (RAS) that mediates extracellular volume and arterial vasoconstriction. It regulates blood pressure and electrolyte homoeostasis. Angiotensin II constricts blood vessels leading to increased blood pressure. It also increases the secretion of ADH and aldosterone, and stimulates the hypothalamus to activate the thirst reflex. An over-active renin-angiotension system leads to vasoconstriction and retention of sodium and water. Renin has been identified to be an attractive target for the treatment of hypertension.

Amplite™ Fluorimetric Renin Assay Kit (Cat# 13530) provides a convenient assay for high throughput screening of renin inhibitors and renin activity using our proprietary TF3/TQ3 fluorescence resonance energy transfer (FRET) peptide. In the FRET peptide, the fluorescence of TF3 is quenched by TQ3. Upon cleavage into two separate fragments by renin, the fluorescence of TF3 is recovered, and the fluorescence signal can be easily monitored by a fluorescence microplate reader at Ex/Em = 540/590 nm. This assay is about fifty fold more sensitive than an EDANS/DABCYL-based assay. With Amplite™ Fluorimetric Renin Assay Kit, we have detected as little as 1ng renin in a 100 µL reaction volume.

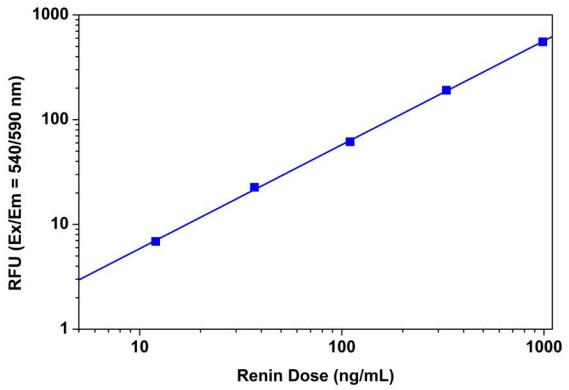

Figure 3.10. Renin dose responses were measured with Amplite™ Fluorimetric Renin Assay Kit (Cat# 13530) on a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.01 ug/mL renin was detected with 30 minutes incubation (n=3).

Table 3.11 Renin Assay Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13530	Amplite™ Fluorimetric Renin Assay Kit *Red Fluorescence*	100 tests	545	576

3.6 Other Protease Assays

Peptidases and proteases play essential roles in protein activation, cell regulation and signaling, as well as in the generation of amino acids for protein synthesis or utilization in other metabolic pathways. In general, peptidases cleave shorter peptides, and proteases cleave longer peptides and proteins. Depending on their sites of cleavage, peptidases can be classified as exopeptidases if they preferentially hydrolyze amino acid residues from the terminus of a peptide, or endopeptidases if they cleave internal peptide bonds. Exopeptidases are further divided into aminopeptidases and carboxypeptidases depending on whether they hydrolyze residues from the amine or the carboxy terminus.

Although the spectral properties of fluorogenic peptidase and protease substrates and their hydrolysis products are easily predictable, the utility of a given substrate for an enzyme depends on the kinetics of hydrolysis by the enzyme, which, in turn, depends on the substrate's concentration and amino acid sequence, as well as on the pH, temperature and presence of cofactors in the medium.

7-Amino-4-methylcoumarin (AMC) is a blue-fluorescent dye whose peptide amides are used extensively as substrates for detecting enzymatic activity in cells, homogenates and solutions. 7-Amino-4-trifluoromethylcoumarin (AFC) is a dye with somewhat longer-wavelength spectra than AMC (excitation/emission maxima of ~380/500 nm) at pH 7. AAT Bioquest offers a variety of substrates based on AMC and AFC.

Rhodamine 110 (R110) is a visible light–excitable dye with much stronger absorption than AMC. R110-based substrates usually comprise two identical amino acids or peptides attached to a single fluorophore. AAT Bioquest's bisamide derivatives of rhodamine 110 are sensitive and selective substrates for assaying protease activity in solution or inside live cells. These substrates comprise an amino acid or peptide covalently linked to each of R110's amino groups, thereby suppressing both its visible absorption and fluorescence. Upon enzymatic cleavage, the nonfluorescent bisamide substrate is converted in a two-step process first to the fluorescent monoamide and then to the even more fluorescent R110. The fluorescence intensities of the monoamide and of R110 are constant from pH 3–9. Both of these hydrolysis products exhibit spectral properties similar to those of fluorescein, with peak excitation and emission wavelengths of 496 nm and 520 nm, respectively, making them compatible with flow cytometers and other instrumentation based on the argon-ion laser. Substrates based on R110 may also be useful for sensitive spectrophotometric assays because the R110 dye has intense visible absorption (extinction coefficient at 496 nm = ~80,000 $cm^{-1}M^{-1}$ in pH 6 solution).

Table 3.12 Other Protease Assay Reagents

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13458	Ala-AMC	5 mg	351	430
13461	BOC-Val-Pro-Arg-AMC	5 mg	351	430
13462	$(BOC-VPR)_2R110$	1 mg	498	520
13459	D-Ala-AMC	5 mg	351	430
13201	D-VLK-AMC [D-Val-Leu-Lys-AMC]	5 mg	351	430
13450	Gly-Pro-AMC	5 mg	351	430
13460	MeO-Succ-Arg-Pro-Tyr-AMC	5 mg	351	430
13457	Pro-AMC	5 mg	351	430
13478	Z-Arg-AMC	5 mg	351	430
13477	Z-Gly-Pro-AMC	5 mg	351	430
13000	Z-Leu-Gly-Arg-Aminoluciferin	1 mg	N/A	560

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3.7 Tide Fluor™ Dyes, Optimized to Develop FRET Protease Assays

Although EDANS, FAM, TAMRA, ROX, Cy 3® and Cy5® have been widely used to develop a variety of FRET peptides and FRET oligonucleotide probes, there are still some limitations in the use of these dyes. For example, the weak absorption and environment-sensitive fluorescence of EDANS has severely limited its sensitivity for developing protease assays and nucleic acid detection probes. Compared to EDANS, fluorescein-based probes (such as FAM, HEX, JOE and TET) have stronger absorption and fluorescence. However the fluorescence of fluorescein-based probes is strongly dependent on pH. They only exhibit the strongest fluorescence at higher pH. This pH dependence makes the fluorescein-based fluorescent probes inconvenient for the assays that require low pH. In addition, most of fluorescein-based probes have quite low photostability, which limits their applications in fluorescence imaging.

Among cyanine dyes, non-sulfonated Cy3® and Cy5® are widely used for developing a variety of peptide and oligonucleotide probes, but they have quite low fluorescence quantum yield in aqueous media. The sulfonated Cy3® and Cy5® have improved fluorescence quantum yield than those of non-sulfonate cyanines. Alexa Fluor™ dyes have improved performance, but are extremely expensive, thus are unpractical for preparing some peptide and oligonucleotide conjugates in some cases.

To address these limitations, AAT Bioquest has developed Tide Fluor™ donor dyes that are optimized as building blocks for developing FRET peptides and oligonucleotides for a variety of biological applications. Our Tide Fluor™ dyes (such as TF1, TF2, TF3, TF4, TF5, TF6, TF7 and TF8) have stronge fluorescence and good photostability. Our TF2 has the similar excitation and emission wavelengths to those of carboxyfluoresceins (FAM), making them readily used for the biological applications that were done with fluoresceins, but have enhanced performance with our TF2 probes. Compared to FAM probes, TF2 has much stronger fluorescence at physiological conditions, and it is much more photostable. Compared to other fluorescent dyes alternative to fluoresceins and Cy dyes (such as Alexa Fluor™ and DyLight™ dyes), Tide Fluor™ dyes are much more cost-effective with comparable or even better performance for your desired biological applications. On Oligonucleotides TF3 are much brighter and more photostable than Cy3®, Alexa Fluor® 555 and DyeLight™ 555 although these dyes have almost identical spectra . We recommend you try our Tide Fluor™ dyes that are optimized for labeling oligonucleotides at much lower cost with comparable performance to Alexa Fluor® dyes. Tide Fluor™ dyes have almost identical spectral properties to Alexa Fluor® dyes as discussed below.

Key Features of Tide Fluor™ Dyes

- *Optimized to pair with Tide Quencher™ dark acceptors*
- *Stronger fluorescence intensity to enhance assay sensitivity*
- *pH-insensitive and environment-insensitive fluorescence*
- *Higher photostability to improve fluorescence imaging*
- *Adjustable water solubility*
- *A variety of reactive forms available for conjugations*

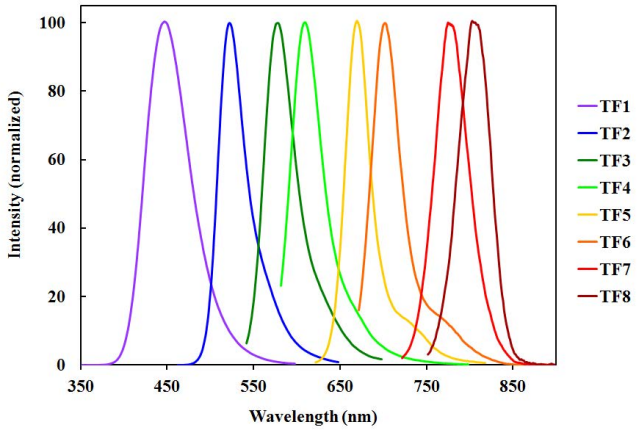

Figure 3.11. The normalized fluorescence spectra of Tide Fluor™ dyes.

Table 3.13 Tide Fluor™ Dye Equivalents of Common Dyes

If you are using	Try this Tide Fluor™ dye
Alexa Fluor® 350, AMCA, DyLight™ 350	Tide Fluor™ 1 [TF 1]
Alexa Fluor® 488, Cy2®, FITC, DyLight™ 488	Tide Fluor™ 2 [TF 2]
Alexa Fluor® 555, Cy3®, DyLight™ 550, TRITC	Tide Fluor™ 3 [TF 3]
Alexa Fluor® 594, DyLight™ 594, Texas Red®	Tide Fluor™ 4 [TF 4]
Alxea Fluor® 647, Cy5®, DyLight™ 650	Tide Fluor™ 5 [TF 5]
Alexa Fluor® 680, Cy5.5®, IRDye® 700, DyLight™ 680	Tide Fluor™ 6 [TF 6]
Alexa Fluor® 750, Cy7®, DyLight™ 750	Tide Fluor™ 7 [TF 7]
Alexa Fluor® 790, DyLight™ 800, IRDye® 800	Tide Fluor™ 8 [TF 8]

Table 3.14 Tide Fluor™ Dyes for Developing FRET Protease Assays

Tide Fluor™ Donor	Ex (nm)	Em (nm)	Features and Benefits	Ordering Information
Tide Fluor™ 1 (TF1)	345	442	Alternative to EDANS • *Much stronger absorption* • *Much stronger fluorescence* • *Less environmental sensitivity*	Cat# 2236 (TF1 azide, click chemistry) Cat# 2237 (TF1 alkyne, click chemistry) Cat# 2238 (TF1 acid) Cat# 2239 (TF1 amine) Cat# 2242 (TF1 maleimide, SH-reactive) Cat# 2244 (TF1 SE, NH_2-reactive)
Tide Fluor™ 2 (TF2)	500	527	Alternative to FAM, FITC and Alexa Fluor® 488 • *pH-insensitive fluorescence* • *Good photostability*	Cat# 2245 (TF2 acid) Cat# 2246 (TF2 amine) Cat# 2247 (TF2 maleimide, SH-reactive) Cat# 2248 (TF2 SE, NH_2-reactive) Cat# 2252 (TF2 azide, click chemistry) Cat# 2253 (TF2 alkyne, click chemistry)
Tide Fluor™ 2WS (TF2WS)	502	525	Alternative to Alexa Fluor® 488 • *pH-insensitive fluorescence* • *Good photostability*	Cat# 2348 (TF2WS acid) Cat# 2249 (TF2WS SE, NH_2-reactive)
Tide Fluor™ 3 (TF3)	555	584	Alternative to Cy3® and Alexa Fluor® 555 • *Strong fluorescence* • *Good photostability*	Cat# 2254 (TF3 azide, click chemistry) Cat# 2255 (TF3 alkyne, click chemistry) Cat# 2268 (TF3 acid) Cat# 2269 (TF3 amine) Cat# 2270 (TF3 maleimide, SH-reactive) Cat# 2271 (TF3 SE, NH_2-reactive)
Tide Fluor™ 3WS (TF3WS)	555	565	Alternative to Cy3® and Alexa Fluor® 555 • *Strong fluorescence* • *Good photostability*	Cat# 2345 (TF3WS acid) Cat# 2346 (TF3WS SE, NH_2-reactive)
Tide Fluor™ 4 (TF4)	590	618	Alternative to ROX, Texas Red® and Alexa Fluor® 594 • *Strong fluorescence* • *Good photostability*	Cat# 2285 (TF4 acid) Cat# 2286 (TF4 amine) Cat# 2287 (TF4 maleimide, SH-reactive) Cat# 2289 (TF4 SE, NH_2-reactive) Cat# 2300 (TF4 azide, click chemistry) Cat# 2301 (TF4 alkyne, click chemistry)
Tide Fluor™ 5WS (TF5WS)	649	664	Alternative to Cy5® and Alexa Fluor® 647 • *Strong fluorescence* • *Good photostability*	Cat# 2275 (TF5WS azide, click chemistry) Cat# 2276 (TF5WS alkyne, click chemistry) Cat# 2278 (TF5WS, acid) Cat# 2279 (TF5WS amine) Cat# 2280 (TF5WS maleimide, SH-reactive) Cat# 2281 (TF5WS SE, NH_2-reactive)
Tide Fluor™ 6WS (TF6WS)	676	695	Alternative to Cy5.5®, IRDye® 700 and Alexa Fluor® 680 • *Strong fluorescence* • *Photostable*	Cat# 2291 (TF6WS acid) Cat# 2292 (TF6WS amine) Cat# 2293 (TF6WS maleimide, SH-reactive) Cat# 2294 (TF6WS SE, NH_2-reactive) Cat# 2302 (TF6WS azide, click chemistry) Cat# 2303 (TF6WS alkyne, click chemistry)
Tide Fluor™ 7WS (TF7WS)	749	775	Alternative to Cy7® and Alexa Fluor® 750 • *Strong fluorescence* • *Good photostability*	Cat# 2304 (TF7WS azide, click chemistry) Cat# 2305 (TF7WS alkyne, click chemistry) Cat# 2330 (TF7WS acid) Cat# 2331 (TF7WS amine) Cat# 2332 (TF7WS maleimide, SH-reactive) Cat# 2333 (TF7WS SE, NH_2-reactive)
Tide Fluor™ 8WS (TF8WS)	775	807	Alternative to IRDye® 800 • *Stronger fluorescence* • *Higher Photostability*	Cat# 2306 (TF8WS azide, click chemistry) Cat# 2307 (TF8WS alkyne, click chemistry) Cat# 2335 (TF8WS acid) Cat# 2336 (TF8WS amine) Cat# 2337 (TF8WS maleimide, SH-reactive) Cat# 2338 (TF8WS SE, NH_2-reactive)

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3.8 Tide Quencher™ Dyes, Optimized to Maximize FRET Efficiency

Although DABCYL has been used to develop a variety of FRET applications, its low quenching efficiency of longer wavelength dyes (such as fluoresceins, rhodamines and cyanines) has limited its use in the development of sensitive fluorogenic FRET probes. Additionally, the absorption spectrum of DABCYL is environment-sensitive. AAT Bioquest has developed the robust Tide Quencher™ acceptor dyes for the development of longer wavelength FRET probes. These Tide Quencher™ dark FRET acceptors (such as TQ1, TQ2, TQ3, TQ4, TQ5, TQ6 and TQ7) are optimized to pair with our Tide Fluor™ dyes and the classic fluorophores (such as AMCA, EDANS, FAM, TAMRA, HEX, JOE, TET, ROX, Cy3®, Cy5® and Cy7®). Like our Tide Fluor™ donor dyes, our Tide Quencher™ acceptor dyes are much more cost-effective with comparable or even better performance for your desired biological applications than other similar products on the market.

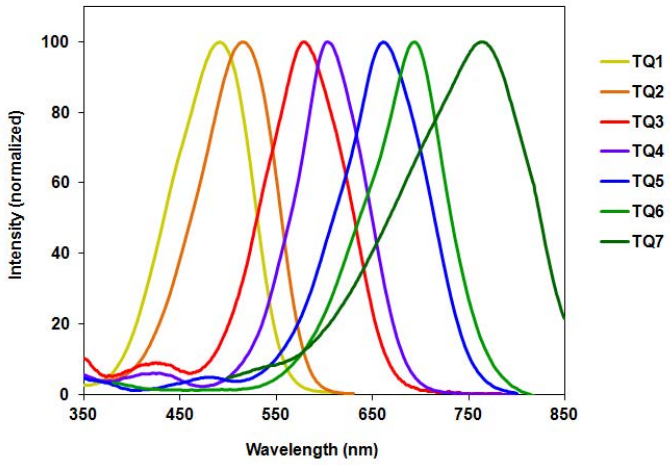

Figure 3.12. The normalized absorption spectra of TQ1, 2, 3, 4, 5, 6 and 7.

Besides their broad applications in the development of Molecular Beacon probes, our Tide Quencher™ dyes have also been used to develop various protease substrates such as HIV protease, MMPs and secretases. In some cases, they have demonstrated greatly improved enzyme performance. This may be partly due to the red-shifted absorption spectrum that overlaps better with the emission spectrum of fluoresceins, rhodamines and cyanines. Tide Quencher™ dyes are a great choice for you to eliminate the limitations of classic quenchers. Tide Quencher ™ dyes are excellent dark quenchers that are individually optimized to pair with all the popular fluorescent dyes such as fluoresceins, rhodamines and cyanines. Our Tide Quencher™ series of nonfluorescent dyes cover the full visible spectrum with unusually high efficiency. TQ2 has absorption maximum perfectly matching the emission of FAM while TQ3, TQ5 and TQ7 are proven to be the best quencher for Cy3®, Cy5® and Cy7®.

The Advantages of Tide Quencher™ Dyes:

- *TQ dyes enable you to explore the FRET potentials that might be impossible with other quenchers.*
- *Versatile reactive forms are convenient for self-constructing your desired FRET biomolecules.*
- *Perfectly match your desired fluorescent donors.*
- *Competitive price with better performance.*

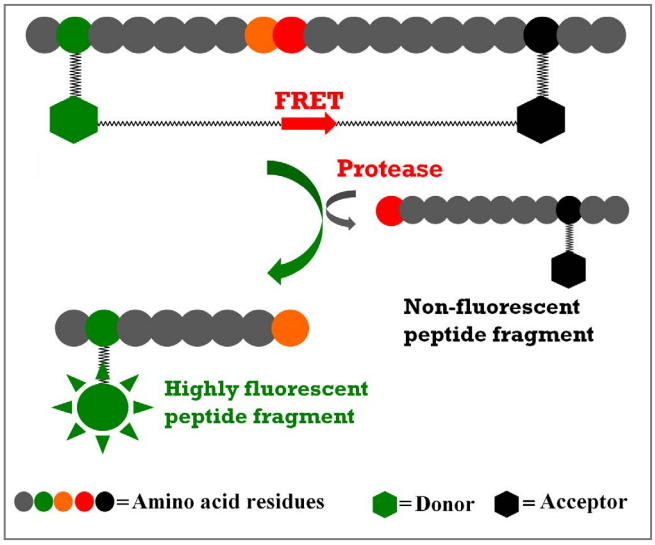

Figure 3.13. The internally quenched FRET peptide substrate is digested by a protease to generate the highly fluorescent peptide fragment. The fluorescence increase is proportional to the protease activity.

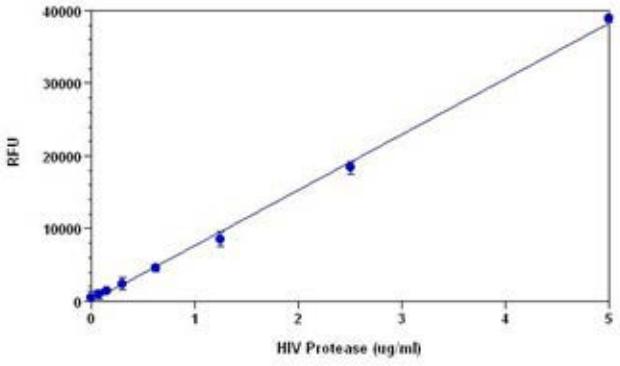

Figure 3.14. HIV Protease cleavage of Arg-Glu(5-FAM)-Val-Ser-Phe-Asn-Phe-Pro-Gln-Ile-Thr-Lys(TQ2)-Arg. The substrate is incubated with HIV protease . Upon HIV protease cleavage, the fluorescence of 5-FAM is recovered and monitored at Ex/Em = 490 /520 nm.

Table 3.15 Tide Quencher™ Dyes for Developing FRET Protease Assays

Dark FRET Acceptor	λ_{max} (nm)	Features and Benefits	Ordering Information
Tide Quencher™ 1 (TQ1)	490	Alternative to Dabcyl, QSY® 35 and BHQ®-0 • *Best paired with Tide Fluor™ 1 (TF1)* • *Excellent FRET efficiency with coumarins*	Cat# 2188 (TQ1 azide, click chemistry) Cat# 2189 (TQ1 alkyne, click chemistry) Cat# 2190 (TQ1 acid) Cat# 2192 (TQ1 amine) Cat# 2193 & 2194 (TQ1 CPG, OH-reactive) Cat# 2196 (TQ1 maleimide, SH-reactive) Cat# 2198 (TQ1 phosphoramidite, OH-reactive) Cat# 2199 (TQ1 SE, NH_2-reactive)
Tide Quencher™ 2 (TQ2)	515	Alternative to BHQ®-1 • *Best paired with Tide Fluor™ 2 (TF2)* • *Better matched with FAM, FITC and Alexa Fluor® 488 than other commercial quenchers*	Cat# 2211 (TQ2 azide, click chemistry) Cat# 2212 (TQ2 alkyne, click chemistry) Cat# 2200 (TQ2 acid) Cat# 2202 (TQ2 amine) Cat# 2203 & 2204 (TQ2 CPG, OH-reactive) Cat# 2206 (TQ2 maleimide, SH-reactive) Cat# 2208 (TQ2 phosphoramidite, OH-reactive) Cat# 2210 (TQ2 SE, NH_2-reactive)
Tide Quencher™ 2WS (TQ2WS)	515	Alternative to BHQ®-1 • *Best paired with Tide Fluor™ 2 (TF2)* • *Better matched with FAM, FITC and Alexa Fluor® 488 than other commercial quenchers*	Cat# 2050 (TQ2WS acid) Cat# 2058 (TQ2WS SE, NH_2-reactive)
Tide Quencher™ 3 (TQ3)	570	Alternative to QSY® 7, QSY® 9 and BHQ®-2 • *Best paired with Tide Fluor™ 3 (TF3)* • *Excellent FRET efficiency with Cy3®, Alexa Fluor® 555 and TAMRA than other commercial quenchers*	Cat# 2220 (TQ3 acid) Cat# 2222 (TQ3 amine) Cat# 2223 & 2224 (TQ3 CPG, OH-reactive) Cat# 2226 (TQ3 maleimide, SH-reactive) Cat# 2228 (TQ3 phosphoramidite, OH-reactive) Cat# 2230 (TQ3 SE, NH_2-reactive) Cat# 2231 (TQ3 azide, click chemistry) Cat# 2232 (TQ3 alkyne, click chemistry)
Tide Quencher™ 3WS (TQ3WS)	578	Alternative to QSY® 7, QSY® 9 and BHQ®-2 • *Best paired with Tide Fluor™ 3 (TF3)* • *Excellent FRET efficiency with Cy3®, Alexa Fluor® 555 and TAMRA than other commercial quenchers*	Cat# 2227 (TQ3WS acid) Cat# 2229 (TQ3WS SE, NH_2-reactive)
Tide Quencher™ 4 (TQ4)	603	• *Strong absorption* • *Best paired with Tide Fluor™ 4 (TF4)* • *Better FRET efficiency with ROX, Texas Red® and Alexa Fluor® 594 than other commercial quenchers*	Cat# 2062 & 2063 (TQ4 CPG, OH-reactive)
Tide Quencher™ 4WS (TQ4WS)	~590	• *Strong absorption* • *Best paired with Tide Fluor™ 4 (TF4)* • *Better FRET efficiency with ROX, Texas Red® and Alexa Fluor® 594 than other commercial quenchers*	Cat# 2060 (TQ4WS acid) Cat# 2061 (TQ4WS amine) Cat# 2064 (TQ4WS maleimide, SH-reactive) Cat# 2067 (TQ4WS SE, NH_2-reactive) Cat# 2068 (TQ4WS azide, click chemistry) Cat# 2069 (TQ4WS alkyne, click chemistry)
Tide Quencher™ 5 (TQ5)	~670	Alternative to QSY® 21 and BHQ®-3 • *Best paired with Tide Fluor™ 5 (TF5)* • *Excellent FRET efficiency with Cy5®, DyLight® 649 and Alexa luor® 647*	Cat# 2077 & 2078 (TQ5 CPG, OH-reactive)
Tide Quencher™ 5WS (TQ5WS)	~670	Alternative to QSY® 21 and BHQ®-3 • *Best paired with Tide Fluor™ 5 (TF5)* • *Excellent FRET efficiency with Cy5®, DyLight® 649 and Alexa Fluor® 647*	Cat# 2075 (TQ5WS acid) Cat# 2076 (TQ5WS amine) Cat# 2079 (TQ5WS maleimide, SH-reactive) Cat# 2081 (TQ5WS SE, NH_2-reactive) Cat# 2082 (TQ5WS azide, click chemistry) Cat # 2083 (TQ5WS alkyne, click chemistry)
Tide Quencher™ 6WS (TQ6WS)	~700	• *Stronger absorption* • *Best paired with Tide Fluor™ 6 (TF6)* • *Better FRET efficiency with Cy5.5®, IRDye® 700 and Alexa Fluor® 680 than other commercial quenchers*	Cat# 2090 (TQ6WS acid) Cat# 2091 (TQ6WS amine) Cat# 2094 (TQ6WS maleimide, SH-reactive) Cat# 2096 (TQ6WS SE, NH_2-reactive) Cat# 2097 (TQ6WS azide, click chemistry) Cat# 2098 (TQ6WS alkyne, click chemistry)
Tide Quencher™ 7WS (TQ7WS)	~760	• *Stronger absorption* • *Best paired with Tide Fluor™ 7 (TF7)* • *Better FRET efficiency with Cy7® and Alexa Fluor® 750 than other commercial quenchers*	Cat# 2105 (TQ7WS acid) Cat# 2106 (TQ7WS amine) Cat# 2109 (TQ7WS maleimide, SH-reactive) Cat# 2111 (TQ7WS SE, NH_2-reactive) Cat# 2112 (TQ7WS azide, click chemistry) Cat# 2113 (TQ7WS alkyne, click chemistry)

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		Blue	Green	Red	
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GTPase	21600, 21659, 21665			21660	
Acetylcholinesterase	11400		11401	11402, 11403	
Galactosidase		14003, 14010	12601, 14001	14019, 14021	12513
Glucosidase		14014			
Glucuronidase		14016			
Cellulase			14025		
Neuraminidase		12602			
Phosphatase	11619, 11950	11610, 11628, 11952	11600, 11630	11629, 11954	11956, 12512
Sphingomyelinase		13620		13621, 13622, 13625	
Phosphodiesterase (PDE)			13602, 13604	13603, 13605, 13640	

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Hydrolytic Enzymes (Other than Proteases)

4.1 ATPases and GTPases

ATPases (EC 3.6.1.3) are a class of enzymes that catalyze the decomposition of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphate ion. This dephosphorylation reaction releases energy, which the enzyme (in most cases) harnesses to drive other chemical reactions that would not otherwise occur. This process is widely used in all known forms of life. Some such enzymes are integral membrane proteins (anchored within biological membranes), and move solutes across the membrane, typically against their concentration gradient. There are different types of ATPases, which can differ in function (ATP synthesis and/or hydrolysis), structure (F-, V- and A-ATPases contain rotary motors) and in the type of ions they transport.

- F-ATPases (F1FO-ATPases) in mitochondria, chloroplasts and bacterial plasma membranes are the prime producers of ATP, using the proton gradient generated by oxidative phosphorylation (mitochondria) or photosynthesis (chloroplasts).
- V-ATPases (V1VO-ATPases) are primarily found in eukaryotic vacuoles, catalyzing ATP hydrolysis to transport solutes and lower pH in organelles like proton pump of lysosome.
- A-ATPases (A1AO-ATPases) are found in Archaea and function like F-ATPases.
- P-ATPases (E1E2-ATPases) are found in bacteria, fungi and eukaryotic plasma membranes and organelles, and function to transport a variety of different ions across membranes.
- E-ATPases are cell-surface enzymes that hydrolyse a range of NTPs, including extracellular ATP.

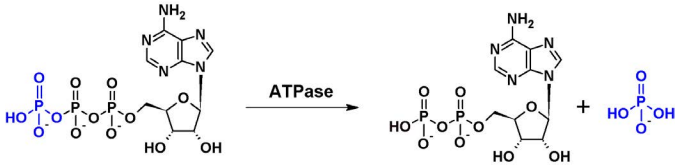

Figure 4.1. ATP Hydrolysis is catalyzed by ATPases.

GTPases are a large family of hydrolase enzymes that can bind and hydrolyze guanosine triphosphate (GTP). The GTP binding and hydrolysis takes place in the highly conserved G domain common to all GTPases. GTPases play an important role in:

- *Signal transduction at the intracellular domain of transmembrane receptors, including recognition of taste, smell and light.*
- *Protein biosynthesis at the ribosome.*
- *Control and differentiation during cell division.*
- *Translocation of proteins through membranes.*
- *Transport of vesicles within the cell. (GTPases control assembly of vesicle coats).*

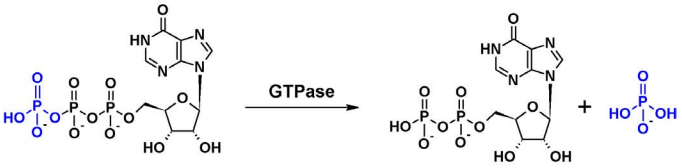

Figure 4.2. GTP Hydrolysis is catalyzed by GTPases

As shown in Figures 4.1 and 4.2, detection of phosphate is one of the most convenient methods for monitoring ATPases and GTPases. Phosphate (Pi) is one of the most important inorganic ions in biological systems. It functions in a variety of roles. One of the most important roles is as a molecular switch, turning enzyme activity on and off through the mediation of the various protein kinases and phosphatases in biological systems. Numerous enzymes of therapeutic relevance produce phosphate directly or through coupled reactions. These potential drug development targets include lipid and protein phosphatases, ATPases, GTPases, prenyltransferases and phosphodiesterases. Phosphate is also of great importance in mineralization processes and is a primary stimulus of algal blooms frequently found in bodies of fresh water, due to run-off from areas of high fertilizer use. The importance of phosphate in drug discovery and other fields makes high quality phosphate assays indispensable.

AAT Bioquest offers a variety of phosphate detection reagents and assay kits. These robust phosphate detection reagents can be conveniently used to assay ATPase and GTPase activities and screening their inhibitors in a HTS mode. PhosphoWorks™ Luminometric ATP Assay Kits can also be used for detecting ATPases. The ATP determination kit is designed to provide a convenient method for the sensitive bioluminescence-based detection of ATP with recombinant firefly luciferase and its substrate luciferin. This assay is based on luciferase's absolute requirement for ATP to produce light. In the presence of Mg^{2+}, luciferase catalyzes the reaction of luciferin, ATP and O_2 to form oxyluciferin, AMP, CO_2, pyrophosphate and ~560 nm light. The luciferin–luciferase bioluminescence assay is extremely sensitive. Most luminometers can detect as little as 1 picomole of ATP as it is consumed or generated in kinetic systems. The high sensitivity of the bioluminescence assay has led to its widespread use for detecting ATP in various enzymatic reactions.

In the presence of inorganic phosphate, MESG is converted to 2-amino-6-mercapto-7-methlpurine by purine nucleoside phosphorylase (pNP) with a red shift of absorption wavelength. This feature has been used to quantify phosphate spectrophotometrically. The enzymatic removal of the ribose moiety from MESG results in a shift in the wavelength of maximum absorbance from 330 nm to 360 nm. Because conversion of MESG requires inorganic phosphate, the increase in absorbance at 360 nm can be used to measure phosphate concentration. When the PNP enzyme and MESG substrate are in excess relative to phosphate, the increase in absorbance at 360 nm is quantitative for inorganic phosphate. Assuming there is no preexisting phosphate, any increase in the absorbance at 360 nm must be the result of phosphate liberation from ATP hydrolysis.

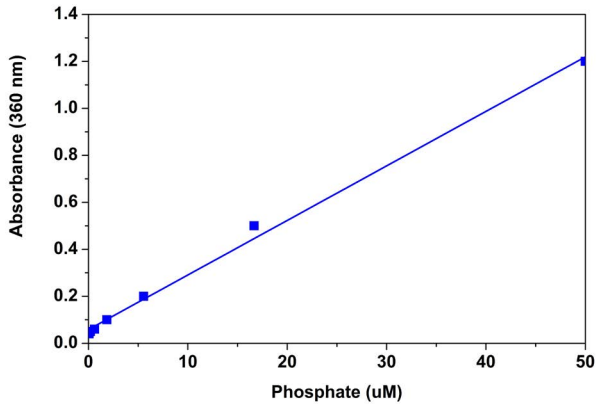

Figure 4.3. Phosphate dose responses were measured with PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit (Cat# 21659) in a 96-well UV plate. As low as 0.2 µM phosphate can be detected with 30 minutes incubation.

PhosphoWorks™ Colorimetric Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme through the complexation of malachite green (MG) with phosphate under acidic conditions. The measurement of Pi is based on the change in MG absorbance in the presence of molybdate. This assay kit is formulated to give sensitive detection of Pi.

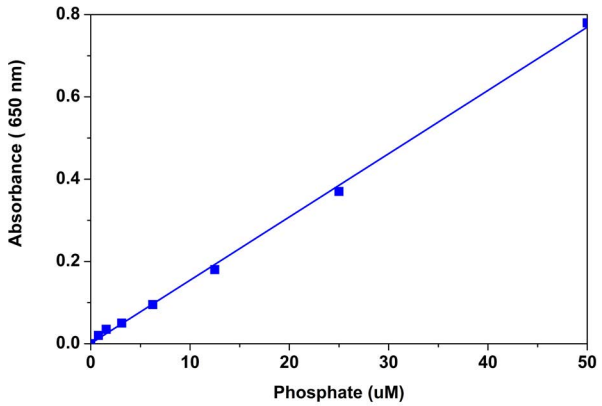

Figure 4.4. Phosphate dose responses were measured with PhosphoWorks™ Colorimetric Phosphate Assay Kit (Cat# 21665) in a 96-well clear plate. As low as 0.1 µM phosphate can be detected with 10 minutes incubation.

Detection of many phosphoester–metabolizing enzymes is difficult because suitable substrates are not available. It usually has been necessary to determine inorganic phosphate release using tedious colorimetric assays or radioisotope-based methods. AAT Bioquest has developed PhosphoWorks™ Fluorimetric Phosphate Assay Kit (Cat# 21660) as an alternative to hazardous radioactive methods and other less sensitive colorimetric assays. The kit measures the activity of any Pi-generating enzyme using our red fluorescent phosphate sensor. The measurement of Pi is based on the change in absorbance and fluorescence of the phosphate sensor. The assay is shown to quantitate phosphate in solution at concentrations as low as 0.1 µM. It can be used to measure the kinetics of phosphate release from phosphatases (such as GTPases and ATPases) by coupling the two enzymatic reactions. It comes with all the essential reagents including phosphate sensor, phosphate standards and assay buffer. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. The assay might be used to monitor the enzyme activities that induce phosphate formation, e.g. ATPases.

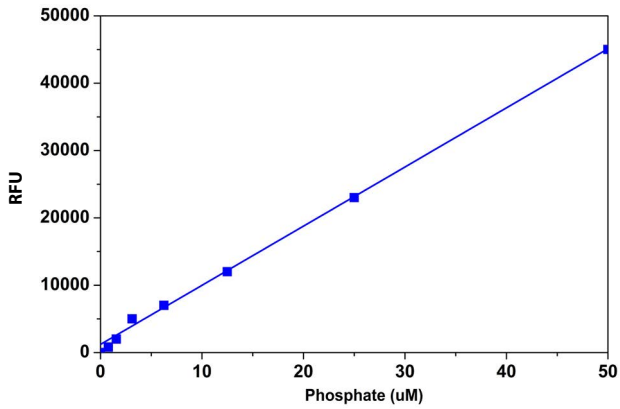

Figure 4.5. Phosphate dose responses were measured with PhosphoWorks™ Fluorimetric Phosphate Assay Kit (Cat# 21660) in a 96-well black plate. The fluorescence was monitored at Ex/Em = 560/590 nm. As low as 0.1 µM phosphate can be detected with 1 hour incubation.

Table 4.1 Phosphate Assay Reagents and Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
8B0458	ATPase(Ab-16) Antibody	50 µg	N/A	N/A
8A0458	ATPase(Phospho-Ser16) Antibody	50 µg	N/A	N/A
21600	MESG *Phosphate Assay Reagent*	5 mg	330	N/A
21659	PhosphoWorks™ Colorimetric MESG Phosphate Assay Kit *UV Absorption*	200 tests	360	N/A
21665	PhosphoWorks™ Colorimetric Phosphate Assay Kit *Blue Color*	1000 tests	650	N/A
21660	PhosphoWorks™ Fluorimetric Phosphate Assay Kit *Red Fluorescence*	125 tests	571	585
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	1 plate	N/A	560
21612	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	1 plate	N/A	560
21613	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	10 plates	N/A	560

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4.2 Acetylcholinesterase

Acetylcholinesterase (AChE) is one of the most crucial enzymes for nerve response and function. AChE degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate the synaptic transmission. AChE inhibitors are among the key drugs approved for Alzheimer's disease (AD) and myasthenia gravis.

Amplite™ Fluorimetric Acetylcholinesterase Assay Kits provide one of the most sensitive methods for detecting AChE activity or screening AChE inhibitors in red florescence window. Kit 11402 uses Amplite™ Red to quantify the choline produced from the hydrolysis of acetylcholine by AChE through choline oxidase-mediated enzyme coupling reactions. The fluorescence intensity of Amplite™ Red is used to measure the amount of choline formed, which is proportional to the AChE activity. It can be used for monitoring and quantifying the AChE activity in blood, cell extracts or other solutions. The kit is an optimized "mix and read" assay that provides a simple one-step fluorimetric assay to detect as little as 0.01 mU AChE in a 100 µL assay volume (0.1 mU/mL). Its signal can be easily read at Ex/Em = ~540/590 nm.

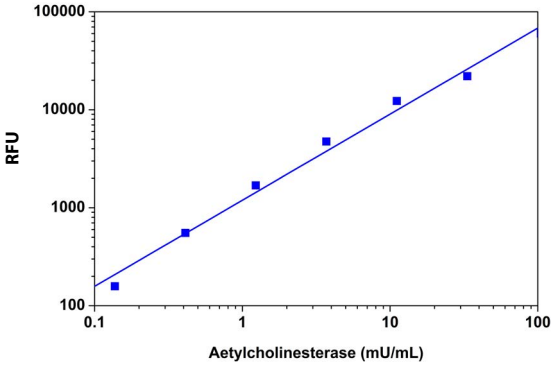

Figure 4.6. Acetylcholinesterase dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Acetylcholinesterase Assay Kit (Cat# 11402). As low as 0.01 mU/well (0.1mU/mL) of acetylcholinesterase was detected with 20 minutes incubation (n=3).

Amplite™ Fluorimetric Acetylcholinesterase Assay Kit (Cat# 11401) uses our outstanding Thiolite™ Green to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE. Thiolite™ Green is not fluorescent until reacted with a thiol group. It has spectral properties similar to those of fluorescein, making this assay compatible with almost every fluorescence instrument. The fluorescence intensity of Thiolite™ Green is used to measure AChE activity. Compared to the existing thiol probes (e.g., mBBr and bBBr), Thiolite™ Green is much more sensitive. This Amplite™ Fluorimetric Acetylcholinesterase Assay Kit provides an ultrasensitive fluorometric one-step assay to detect as little as 0.01mU AChE in a 100 µL assay volume (0.1 mU/mL). Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

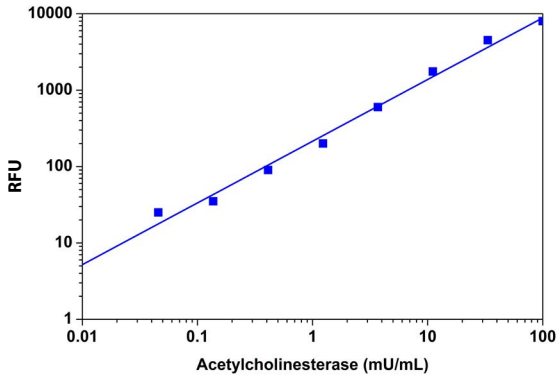

Figure 4.8. Acetylcholinesterase dose responses were measured in a 96-well black solid plate with Kit 11401. As low as 0.01 mU/well of acetylcholinesterase can be detected.

Amplite™ Colorimetric Acetylcholinesterase Assay Kit (Cat# 11400) uses DTNB to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE in blood, in cell extracts, and in other solutions. The absorption intensity of DTNB adduct is used to measure the amount of thiocholine formed, which is proportional to the AChE activity. The kit provides a colorimetric one-step assay to detect as little as 0.1 mU AChE in a 100 µL assay volume (1 mU/mL). Its signal can be easily read at ~410 nm.

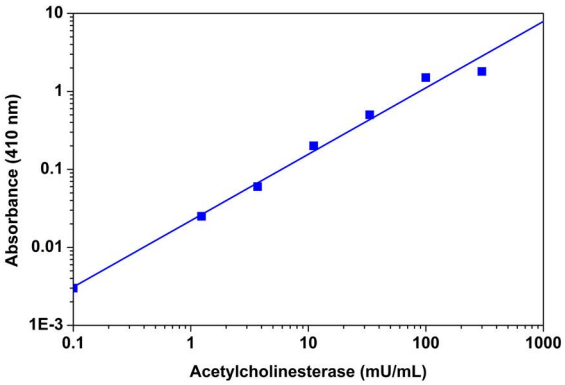

Figure 4.7. Acetylcholinesterase dose responses were measured in a 96-well clear plate with Kit 11400. As low as 0.1 mU/well of acetylcholinesterase can be detected.

Table 4.2 Acetylcholinesterase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
40007	Amplite™ Choline Quantitation Kit	200 tests	571	585
11400	Amplite™ Colorimetric Acetylcholinesterase Assay Kit	200 tests	410	N/A
11403	Amplite™ Fluorimetric Acetylcholine Assay Kit *Red Fluorescence*	200 tests	571	585
11401	Amplite™ Fluorimetric Acetylcholinesterase Assay Kit *Green Fluorescence*	200 tests	510	524
11402	Amplite™ Fluorimetric Acetylcholinesterase Assay Kit *Red Fluorescence*	200 tests	571	585

4.3 Glycosidases

Glycosidase enzymes exhibit very high selectivity for hydrolysis of their preferred sugars. For example, β-galactosidase rapidly hydrolyzes β-D-galactopyranosides but usually does not hydrolyze either the anomeric α-D-galactopyranosides or the isomeric β-D-glucopyranosides. Endogenous glycosidase activity is frequently used to characterize strains of microorganisms and to selectively label organelles of mammalian cells. Defects in glycosidase activity are characteristic of several diseases. In addition, glycosidases are important reporter gene markers. Specifically, lacZ, which encodes β-galactosidase, is extensively used as a reporter gene in animals and yeast, whereas the β-glucuronidase (GUS) gene is a popular reporter gene in plants. Glycosidase substrates are also used in conjunction with glycosidase-conjugated secondary detection reagents in immunohistochemical techniques and enzyme-linked immunosorbent assays.

The fluorogenic β-galactosidase substrate 4-methylumbelliferyl β-D-galactopyranoside (MUG) is commonly used to detect β-galactosidase activity in cell extracts, lysosomes and human blood serum. MUG is one of the most common fluorogenic galactosidase substrates. However, the hydrolysis product, β-methylumbelliferone, has a relatively high pKa (~7.8), precluding its use for continuous measurement of enzymatic activity. Similar to MUG, 3-carboxyumbelliferyl beta-D-galactopyranoside(CUG) has also been used for detecting galactosidases. CUG carries a carboxy group, thus might be conjugated to a carrier molecule some highly selective detection of galactosidase.

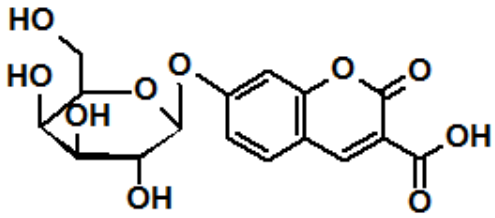

Figure 4.9. The chemical structure of CUG [3-Carboxyumbelliferyl beta-D -galactopyranoside].

Fluorescein di-β-D-galactopyranoside (FDG) might be the most sensitive fluorogenic substrate for detecting β-galactosidase. Nonfluorescent FDG is sequentially hydrolyzed by β-galactosidase, first to fluorescein monogalactoside and then to highly fluorescent fluorescein. Enzyme-mediated hydrolysis of FDG can be followed by the increase in either absorbance or fluorescence. Although the turnover rates of FDG and its analogs are considerably slower than that of the common spectrophotometric galactosidase substrate, o-nitrophenyl β-D-galactopyranoside (ONPG), the absorbance of fluorescein is about fivefold greater than that of o-nitrophenol. Moreover, fluorescence-based measurements can be several orders of magnitude more sensitive than absorption-based measurements.

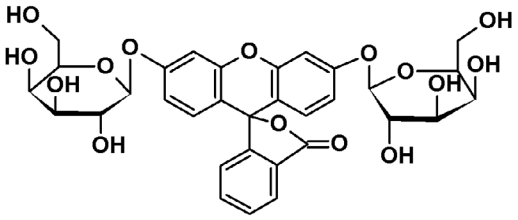

Figure 4.10. The chemical structure of FDG [Fluorescein di-beta-D-galactopyranoside].

Unlike FDG, resorufin β-D-galactopyranoside requires only a single-step hydrolysis reaction to attain full fluorescence. The relatively low pKa (~6.0) of its hydrolysis product, resorufin, permits its use for continuous measurement of enzymatic activity at physiological pH. Resorufin galactoside has also been used to quantitate β-galactosidase activity in single yeast cells by flow cytometry, and to detect immobilized β-galactosidase activity in bioreactors.

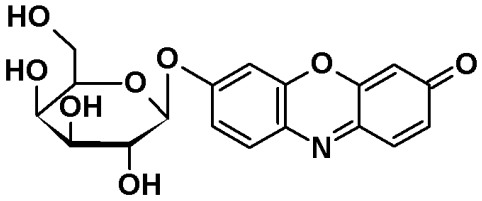

Figure 4.11. The chemical structure of resorufin beta-D-galactopyranoside.

Table 4.3 Glycosidase Assay Reagents and Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)	Function
12601	Amplite™ Fluorimetric Beta-Galactosidase Assay Kit *Green Fluorescence*	500 tests	490	514	β-galactosidase detection
14003	CUG [3-Carboxyumbelliferyl beta-D-galactopyranoside]	10 mg	386	448	β-galactosidase detection
12513	D-Luciferin Galactoside	5 mg	328	533	β-galactosidase detection
14025	FCB [Fluorescein di-beta-D-cellobioside]	1 mg	490	514	Cellulase detection
14001	FDG [Fluorescein di-beta-D-galactopyranoside]	5 mg	490	514	β-galactosidase detection
14002	FDGlcU [Fluorescein di-beta-D-glucuronide]	1 mg	490	514	β-glucuronidase detection
14014	4-Methylumbelliferyl-beta-D-glucoside *UltraPure Grade*	25 mg	360	449	β-glucosidase detection
14010	MUG [4-Methylumbelliferyl-beta-D-galactopyranoside] *UltraPure grade*	25 mg	360	449	β-galactosidase detection
14016	MUGlcU [4-Methylumbelliferyl-beta-D-glucuronide]	5 mg	360	449	β-glucuronidase detection
14021	Resorufin Alpha-D-galactopyranoside	5 mg	571	585	α-galactosidase detection
14019	Resorufin Beta-D-galactopyranoside	5 mg	571	585	β-galactosidase detection

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D-luciferin galactoside contains a beta-galactoside moiety attached at the 6-O-position, thus preventing it be recognized by the firefly luciferase enzyme. However, the galactose moiety is removed effectively by beta-galactosidase activity, and the resulted D-luciferin is well recognized by luciferase. D-Luciferin galactoside is a sensitive substrate for the chemiluminescent measurement of galactosidase activity in homogeneous assays, or in cell lysate samples in combination with luciferase and its cofactors. When used as a dual substrate, beta-galactosidase (LacZ gene) and luciferase levels can be determined with high sensitivity. When used in a model system expressing firefly luciferase, the luciferin is then utilized in a firefly luciferase reaction to generate light. It can also be used for *in vivo* bioluminescence imaging applications.

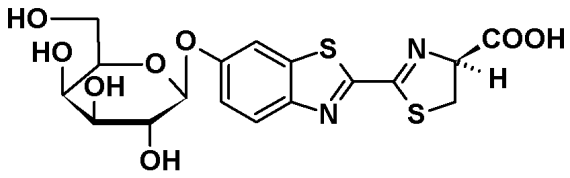

Figure 4.12. The chemical structure of D-luciferin galactoside.

E. coli β-galactosidase is a 464 kD tetramer. Each unit of β-galactosidase consists of five domains, the third of which is the active site. It is an essential enzyme in cells. Deficiencies of this enzyme can result in galactosialidosis or Morquio B syndrome. In E. coli, β-galactosidase is produced by the activation of LacZ operon. Detection of LacZ expression has become routine to the point of detection of as few as 5 copies of β-galactosidase per cell. Amplite™ Fluorimetric Beta-Galactosidase Assay Kit (Cat# 12601) uses the fluorogenic fluorescein digalactoside (FDG) galactosidase substrate that can sensitively distinguish LacZ+ from LacZ- cells. The non-fluorescent substate generates the strongly fluorescent fluorescein upon reaction with galactosidase. It can be used either for detecting galactosidase conjugates in ELISA type assay systems or for monitoring LacZ gene expression in cells. FDG used in the kit is not fluorescent. The galactosidase induced cleavage of FDG gives fluorescein that has the spectra of Ex/Em = 490/515 nm, which can be detected with most fluorescence instruments equipped with a FITC filter set. The kit comes with all the essential components with an optimized assay protocol. It can be used with a fluorescence microplate reader, a fluorescence microscope, or a flow cytometer. It might also be used for screening galactosidase inhibitors.

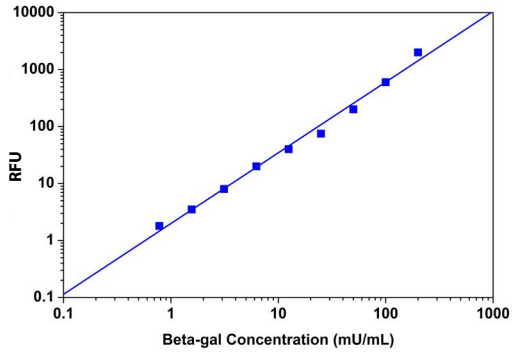

Figure 4.13. ß-galactosidase dose responses were measured with Amplite™ Fluorimetric beta-Galactosidase Assay Kit (Cat# 12601) in a Costar 96-well black solid plate using Gemini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU/well ß-galactosidase was detected with 30 minutes incubation time.

4.4 Neuraminidase Assay

Neuraminidases, also called sialidases, are glycoside hydrolase enzymes that catalyze the hydrolysis of terminal sialic acid residues and neuraminic acids. The most commonly known neuraminidase is the viral neuraminidase. The cleavage of linkage between sialic acid and adjacent sugar residue permits the transport of the virus through mucin and destroys the haemagglutinin receptor on the host cell, thus allowing elution of progeny virus particles from infected cells. There are two major classes of neuraminidase that cleave exo or endo poly-sialic acids:

- Exo hydrolysis of α-(2→3)-, α-(2→6)-, α-(2→8)-glycosidic linkages of terminal sialic acid residues
- Endo hydrolysis of (2→8)-α-sialosyl linkages in oligo- or poly(sialic) acids

Neuraminidase promotes influenza virus release from infected cells and facilitates virus spread within the respiratory tract. Thus, it is an important target for influenza drug development. The detection of neuraminidase and screening its inhibitors is one of the essential tasks for investigating biological processes and preventing influenza infection.

There are a few assay kits available for detecting neuraminidase, but all the commercial available kits are tedious to use. Amplite™ Fluorimetric Neuraminidase Assay Kit (Cat# 12602) provides a sensitive and robust fluorimetric assay to detect neuraminidase that exists either in cells or biological samples. The non-fluorescent neuraminidase substrate becomes strongly fluorescent upon neuraminidase cleavage. The kit can detect as little as 0.3 mU/mL neuraminidase in a 100 µL assay volume. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The signal can be easily read using a fluorescence microplate reader at Ex/Em = ~320/~450 nm.

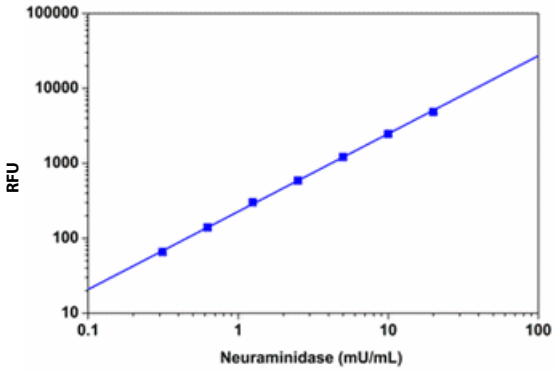

Figure 4.14. Neuraminidase dose responses were measured in a 96-well black plate with Amplite™ Fluorimetric Neuraminidase Assay Kit (Cat# 12602) using a Germini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU/mL of neuraminidase was detected with 1 hour incubation.

Table 4.4 Neuraminidase Assay Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
12602	Amplite™ Fluorimetric Neuraminidase Assay Kit *Blue Fluorescence*	200 tests	360	449

4.5 Phosphatases

Cells utilize a wide variety of phosphate and polyphosphate esters as enzyme substrates, second messengers, membrane structural components and vital energy reservoirs. Aalkaline and acid phosphatase hydrolyze phosphate monoesters to an alcohol and inorganic phosphate. Conjugates of calf intestinal alkaline phosphatase are extensively used as secondary detection reagents in ELISAs, immunohistochemical techniques and Southern, Northern and Western blot analyses. In addition, phosphatases serve as enzyme markers, allowing researchers to identify primordial germ cells to distinguish subpopulations of bone marrow stromal cells and to investigate *in vitro* differentiation in carcinoma cell lines. PALP-1, the gene for human placental alkaline phosphatase, has been used as a eukaryotic reporter gene that is superior to lacZ for lineage studies in murine retina. This gene has also been engineered to produce a secreted alkaline phosphatase, allowing quantitation of gene expression without disrupting the cells.

Alkaline phosphatase (ALP) (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate group from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. An important use of alkaline phosphatase is as a label for enzyme immunoassays. Alkaline phosphatase is a highly sensitive enzyme for ELISA, immuno-histochemical as well as Northern, Southern and Western blot applications. It is widely used in various biological assays (in particular, immunoassays) and ELISA-based diagnostics.

Fluorescein diphosphate (FDP) might be the most sensitive fluorogenic phosphatase substrate available. The colorless and nonfluorescent FDP reagent is hydrolyzed to fluorescein which exhibits superior spectral properties (EC ~90,000 $cm^{-1}M^{-1}$, quantum yield ~0.92). FDP is an excellent substrate for alkaline phosphatase in ELISAs, providing detection limits at least 50 times lower than those obtained with the chromogenic 4-nitrophenyl phosphate.

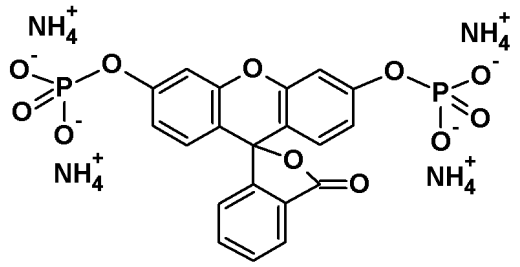

Figure 4.15. The chemical structure of FDP [fluorescein diphosphate].

AAT Bioquest's Sun Red™ phosphate is a fluorogenic phosphatase substrate that yields a red fluorescent hydrolysis product, which is efficiently excited by the 633 nm spectral line of the He-Ne laser to produce bright red fluorescence with absorption/emission maxima of ~646/659 nm. Although the substrate itself is fluorescent, the difference between the substrate's excitation maximum and that of the phenolic hydrolysis product is over 200 nm, allowing the two species to be easily distinguished. Sun Red™ phosphate has good water solubility, a low K_m and a high turnover rate, making it particularly useful for both fluorescence- and absorption-based microplate assays.

4-Methylumbelliferyl phosphate (MUP) is a widely used fluorogenic substrate for alkaline phosphatase detection. MUP has been used for a variety of ELISA protocols in which the relatively high pH optimum of alkaline phosphatase permits continuous detection of the rate of formation of 4-methylumbelliferone (7-hydroxy-4-methylcoumarin). MUP has also been used to count cells based on their alkaline phosphatase activity, to detect PCR amplification products and to identify and characterize bacteria.

Besides MUP, AAT Bioquest also offers CF-MUP, which exhibits extraordinary spectral properties that are advantageous for the assay of both acid and alkaline phosphatase activity. CF-MUP might also be useful for measuring the activity of protein phosphatases that are important for high-throughput screening applications under physiological conditions. The hydrolysis product of CF-MUP exhibits both a lower pKa (4.8 versus 7.8 for MU) and a higher fluorescence quantum yield (0.88 versus 0.63) than the hydrolysis product of MUP. The low pKa of its hydrolysis product makes CF-MUP a sensitive substrate for the continuous assay of acid phosphatases, which is not possible with MUP because its fluorescence must be measured at alkaline.

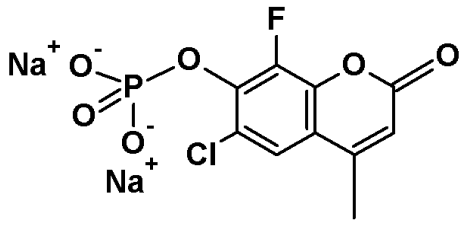

Figure 4.16. The chemical structure of CF-MUP.

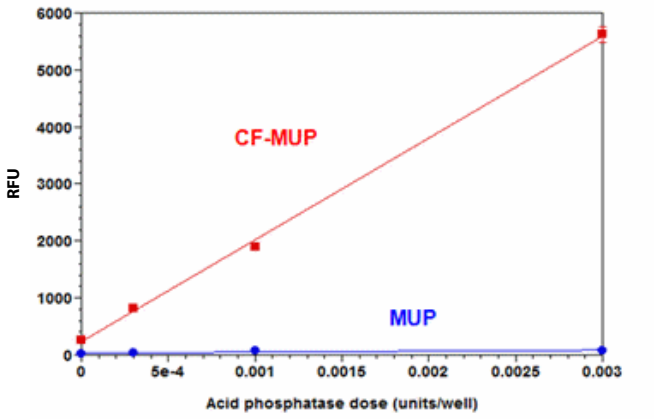

Figure 4.17. Detection of acid phosphatase activity with CF-MUP (Cat# 11628) and MUP (Cat# 11610). The concentrations of the two substrates (initially approximately 10 mM) were matched by normalizing the absorbance of each substrate solution at 319 nm (pH 10) to a value of 0.52 (assuming the extinction coefficient of each substrate was approximately equivalent). The resulting florescence signals were recorded using excitation at 360 nm and emission at 450 nm.

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PhosLite™ Green yields a fluorescent precipitate at the site of phosphatase activity. It is the fluorescence version of 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) phosphatase detection system. Upon enzymatic cleavage, this weakly blue-fluorescent substrate yields an extremely photostable green fluorescent precipitate that is up to 40 times brighter than the signal achieved when using either directly labeled fluorescent hybridization probes or fluorescent secondary detection methods in comparable applications. It can be readily used to detect acid phosphatases, e.g., tartrate-resistant acid phosphatase (TRAP) activity, a marker of osteoclast differentiation from hematopoietic stem cells.

Amplite™ Colorimetric Alkaline Phosphatase Assay Kit 11950 uses *p*NPP, a chromogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions and in cell extracts. The kit provides an optimized "mix and read" assay protocol which is compatible with HTS liquid handling instruments. Its signal can be easily read by an absorbance microplate reader at ~400 nm.

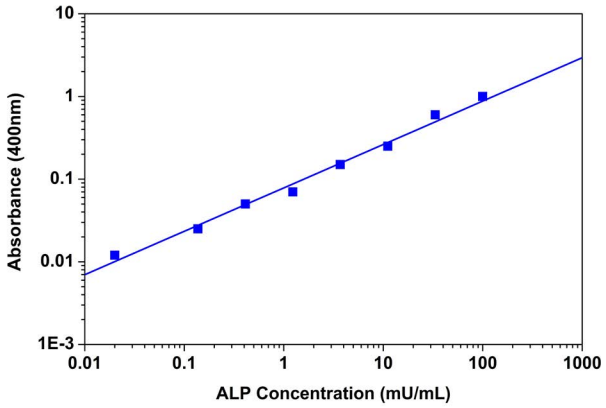

Figure 4.18. Alkaline phosphatase dose responses were measured with Amplite™ Colorimetric Alkaline Phosphatase Assay Kit (Cat# 11950) in a white/clear bottom 96-well plate using a NOVOstar microplate reader (BMG Labtech). As low as 0.3 mU/mL of alkaline phosphatase can be detected with 30 minutes incubation time (n=3).

Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit 11952 uses our MUP Plus™-based coumarin substrate. Similar to MUP, MUP Plus™ is sensitive to phosphatase-induced hydrolysis, giving the halogenated coumarin that possesses intense blue fluorescence. Its almost identical spectral properties to those of MUP enables MUP Plus™ substrates readily compatible with many fluorescence instrument systems equipped with MUP settings. Compared to MUP, MUP Plus™ gives the coumarin fluorophore that has substantially lower pKa, making the MUP Plus™ assay much less pH-dependent. Meanwhile, MUP Plus™, a fluorogenic phosphatase substrate, can quantify alkaline phosphatase activity in solutions and in cell extracts. It can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = ~360/450 nm.

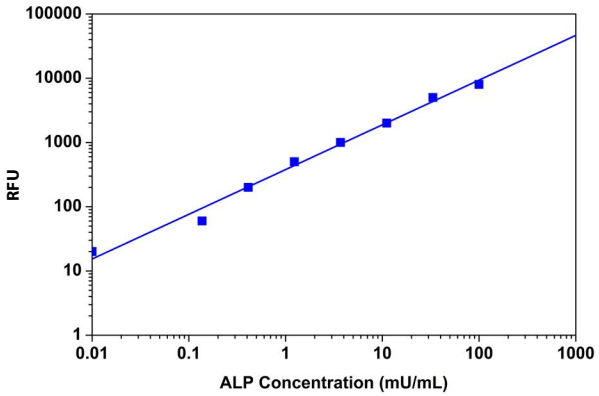

Figure 4.19. Alkaline phosphatase dose responses were measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit (Cat# 11952)in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.1 mU/mL of alkaline phosphatase can be detected with 30 minutes incubation time (n=3).

Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit 11953 uses FDP, a green fluorogenic phosphatase substrate, to quantify the alkaline phosphatase activity in solutions and in cell extracts. It can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = ~490/525 nm.

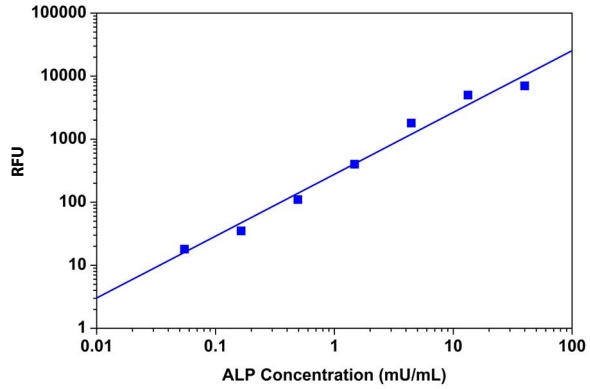

Figure 4.20. Alkaline phosphatase dose responses were measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit (Cat# 11953) in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.1 mU/mL of alkaline phosphatase can be detected with 30 minutes incubation time (n=3).

Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit 11954 uses SunRed™-based substrate. The weakly fluorescent SunRed™ phosphate is sensitive to phosphatase-induced hydrolysis, giving the SunRed™ fluorophore that possesses intense red fluorescence. Upon phosphatase-induced hydrolysis, the SunRed™ phosphate solution has its absorption blue-shifted more than 100 nm. The

maximum absorption of SunRed™ fluorophore at 633 nm makes this substrate an ideal NIR probe that can be readily detected with many fluorescence instrument systems often equipped with Cy5® settings.

Based on the near infrared fluorescence of SunRed™ fluorophore, the signal can be easily read by a fluorescence microplate reader at Ex/Em = ~630/660 nm. The kit has been used for the high throughput screening of protein phosphatase inhibitors due to its low interference from biological sample. It can be performed in a convenient 96-well or 384-well microtiter-plate format.

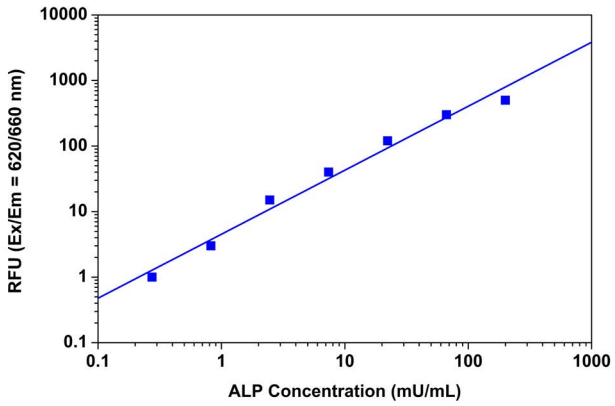

Figure 4.21. Alkaline phosphatase dose responses were measured with Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit (Cat# 11954) in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.3 mU/mL of alkaline phosphatase was detected with 60 minutes incubation (n=3).

Amplite™ Luminometric Alkaline Phosphatase Assay Kit 11956 uses D-luciferin phosphate as the luminogenic phosphatase substrate to quantify alkaline phosphatase activity in solutions and in cells. D-luciferin phosphate is not recognized by luciferase until its phosphate group is removed to give luciferin. The kit provides an optimized "mix and read" assay protocol which is compatible with HTS liquid-handling instruments. Amplite™ Luminometric Alkaline Phosphatase Assay Kit can be readily performed in a 96-well or 384-well microtiter-plate format. Its signal can be easily read by luminescence microplate readers. The high sensitivity makes the kit ideal for the assays that require low detection limit.

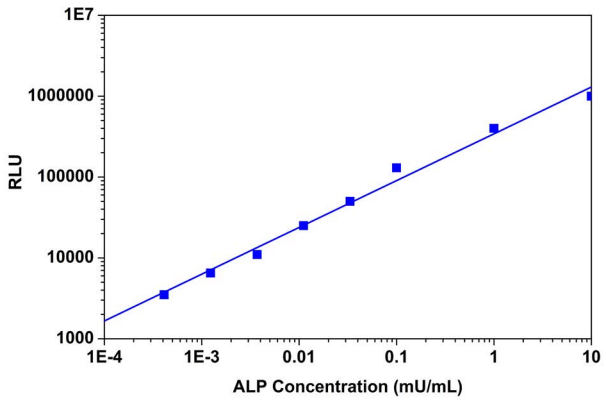

Figure 4.22. Alkaline phosphatase dose responses were measured with Amplite™ Luminometric Alkaline Phosphatase Assay Kit (Cat# 11956) in a 96-well white plate using a NOVOstar microplate reader (BMG Labtech). As low as 0.001 mU/mL of alkaline phosphatase was detected with 20 minutes incubation (n=3).

Table 4.5 Phosphatase Reagents and Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11950	Amplite™ Colorimetric Alkaline Phosphatase Assay Kit *Yellow Color*	500 tests	405	N/A
11952	Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Blue Fluorescence*	500 tests	360	449
11953	Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Green Fluorescence*	500 tests	490	514
11954	Amplite™ Fluorimetric Alkaline Phosphatase Assay Kit *Near Infrared Fluorescence*	500 tests	646	660
11956	Amplite™ Luminometric Alkaline Phosphatase Assay Kit *Luminescence*	100 tests	N/A	560
11628	CF-MUP, Sodium Salt *Superior alternative to MUP*	10 mg	360	450
12512	D-Luciferin Phosphate	1 mg	358	533
11600	FDP [Fluorescein diphosphate, tetraammonium salt]	5 mg	490	514
11610	MUP, Disodium Salt [4-Methylumbelliferyl phosphate, disodium salt] *UltraPure Grade*	25 mg	360	449
11630	PhosLite™ Green	1 mg	345	520
11619	pNPP [4-Nitrophenyl phosphate, disodium salt] *UltraPure Grade*	25 mg	399	N/A
11629	SunRed™ Phosphate	5 mg	646	659

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4.6 Phosphodiesterases

Phosphodiesterases (PDE) is a group of enzymes that degrade the second messenger molecules: cyclic nucleotides cAMP and cGMP. They regulate the localization, duration, and amplitude of cyclic nucleotide signaling within subcellular domains. PDEs are therefore important regulators of signal transduction mediated by these second messenger molecules. Based on amino acid sequences, substrate specificities, regulatory properties, pharmacological properties and tissue distribution, the superfamily of PDE enzymes is classified into 11 families, namely PDE1-PDE11 in mammals.

Different PDEs of the same family are functionally related despite the fact that their amino acid sequences can show considerable divergence. PDEs have different substrate specificities. Some are cAMP-selective hydrolases (PDE4, 7 and 8); others are cGMP-selective (PDE5, 6, and 9). They can hydrolyse both cAMP and cGMP (PDE1, 2, 3, 10, and 11). PDE3 is sometimes referred to as cGMP-inhibited phosphodiesterase. Although PDE2 can hydrolyze both cyclic nucleotides, binding of cGMP to the regulatory GAF-B domain will increase cAMP affinity and hydrolysis to the detriment of cGMP. This mechanism, as well as others, allows for cross-regulation of the cAMP and cGMP pathways.

PDE enzymes are often targets for pharmacological inhibition due to their unique tissue distribution, structural and functional properties. Inhibitors of PDE can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE. PDE inhibitors have been identified as new potential therapeutics in areas such as pulmonary arterial hypertension, coronary heart disease, dementia, depression and schizophrenia.

FAM-cAMP and TAMRA-cAMP derivatives are specific fluorescent substrates for PDE IV with green and red fluorescence respectively. They can be used for assaying PDE IV activities or screening PDE IV inhibitors in combination with anti-cAMP antibody in a FRET readout or FP format. Due to its longer excitation and emission wavelengths, TAMRA-cAMP substrate is preferred for screening PDE IV inhibitors. Similarly, FAM-cGMP and TAMRA-cGMP derivatives are specific fluorescent substrates for PDE V with green and red fluorescence respectively.

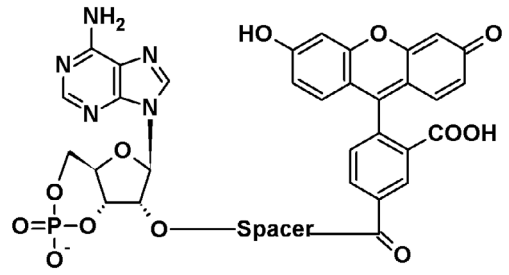

Figure 4.23. The chemical structure of FAM-cAMP PDE IV substrate.

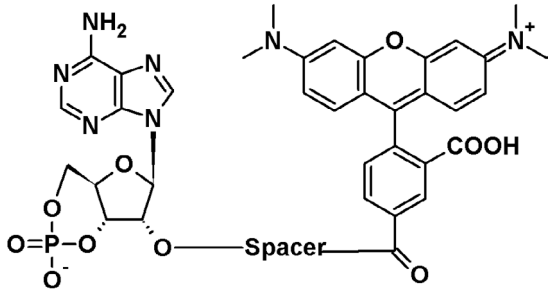

Figure 4.24. The chemical structure of TAMRA-cAMP PDE IV substrate.

Table 4.6 Phosphodiesterase Assay Reagents

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13610	AMP-Fluorescein Conjugate Calibrator	100 nmol	492	515
13602	FAM-cAMP PDE IV Substrate *Green fluorescence*	0.5 umol	492	515
13604	FAM-cGMP PDE V Substrate *Green fluorescence*	0.5 umol	492	515
13611	GMP-Fluorescein Conjugate Calibrator	100 nmol	492	515
11640	Resorufin Phosphocholine	1 mg	575	590
36370	Screen Quest™ Colorimetric ELISA cAMP Assay Kit	1 plate	650	N/A
36373	Screen Quest™ Fluorimetric ELISA cAMP Assay Kit	1 plate	571	585
13603	TAMRA-cAMP PDE IV Substrate *Red fluorescence*	0.5 umol	544	575
13605	TAMRA-cGMP PDE V Substrate *Red fluorescence*	0.5 umol	544	575

4.7 Sphingomyelinases

Sphingomyelinase (SMase) is an enzyme that is responsible for cleaving sphingomyelin (SM) to phosphocholine and ceramide. Activation of SMases in cells plays an important role in the cellular responses. Five types of sphingomyelinase (SMase) have been identified based on their cation dependence and pH optima of action. They are lysosomal acid SMase, secreted zinc-dependent acid SMase, magnesium-dependent neutral SMase, magnesium-independent neutral SMase, and alkaline SMase. Among the five types, the lysosomal acidic SMase and the magnesium-dependent neutral SMase are considered major candidates for the production of ceramide in the cellular response to stress.

Amplite™ Colorimetric Sphingomyelinase Assay Kit (Cat# 13620) provides a sensitive method for detecting neutral SMase activity or screening its inhibitors. The kit uses Amplite™ Blue as a colorimetric probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin by sphingomyelinase (SMase). It can be used for measuring the SMase activity in blood, cell extracts or other solutions. The absorbance of light at 655 nm is proportional to the formation of phosphocholine, therefore to the SMase activity. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments.

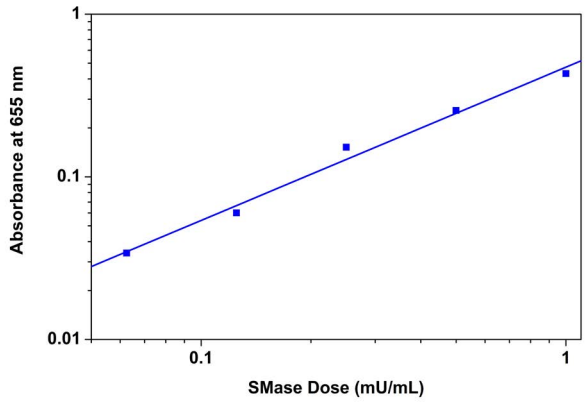

Figure 4.24. Sphingomyelinase dose responses were measured in a 96-well white wall/clear bottom plate with Amplite™ Colorimetric Sphingomyelinase Assay Kit (Cat# 13620) using a Spectrum Max microplate reader (Molecular Devices). As low as 0.08 mU/mL of sphingomyelinase can be detected with 60 minutes incubation time (n=3).

Amplite™ Fluorimetric Sphingomyelinase Assay Kit (Cat# 13621) provides the most sensitive method for detecting neutral SMase activity or screening its inhibitors. The kit uses Amplite™ Red as a fluorogenic probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin by sphingomyelinase (SMase). It can be used for measuring the SMase activity in blood, cell extracts or other solutions. The fluorescence intensity of Amplite™ Red is proportional to the formation of phosphocholine, therefore to the SMase activity. Amplite™ Red enables the assay readable either in fluorescence intensity or absorption mode. The kit is an optimized "mix and read" assay that can be used for real time monitoring of Smase activities. Kit 13622 has been developed for monitoring acid SMase activity.

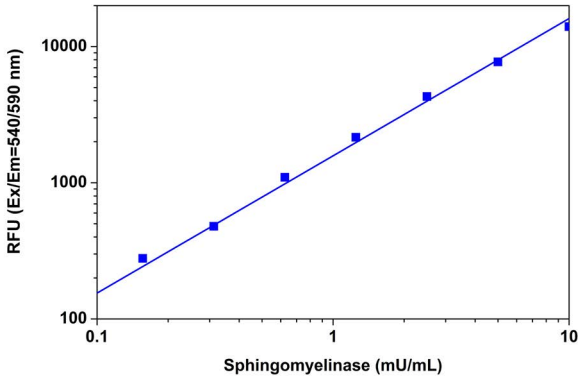

Figure 4.25. Sphingomyelinase dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Sphingomyelinase Assay Kit (Cat# 13621) using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.15 mU/mL of sphingomyelinase can be detected with 60 minutes incubation time (n=3).

Table 4.7 Sphingomyelinase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13620	Amplite™ Colorimetric Sphingomyelinase Assay Kit *Blue Color*	200 tests	655	N/A
13622	Amplite™ Fluorimetric Acidic Sphingomyelinase Assay Kit *Red Fluorescence*	200 tests	571	585
13625	Amplite™ Fluorimetric Sphingomyelin Assay Kit *Red Fluorescence*	100 tests	571	585
13621	Amplite™ Fluorimetric Sphingomyelinase Assay Kit *Red Fluorescence*	200 tests	571	585

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Oxidases 5

oxidase probes and assay kits at-a-glance*

Enzyme	Colorimetric	Fluorimetric		Luminometric
		Blue	Red	
Catalase			11306	
Cytochrome P450		15001, 15005	15020, 15023, 15024, 15025	
Glucose Oxidase			11300	
Glutamate Oxidase			11302	
Horseradish Peroxidase (HRP)	11001, 11005, 11551		11000, 11011, 11540, 11541, 11552, 11553	11559
Lysyl Oxidase (LOX)			15255	
Monoamine Oxidase (MAO)			11303, 11501, 11502	
Myeloperoxidase			11301	
Superoxide Dismutase (SOD)			11305	
Xanthine Oxidase			11304	

* products listed by catalog number

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Oxidases

5.1 Catalase

Catalase is a common antioxidant heme-containing redox enzyme found in nearly all living organisms that are exposed to oxygen. The enzyme is concentrated in the peroxisome subcellular organelles. Hydrogen peroxide is a toxic product of normal aerobic metabolism and pathogenic ROS production involving oxidase and superoxide dismutase reactions. By preventing the excessive buildup of H_2O_2, catalase allows important cellular processes which produce H_2O_2 as a by-product to take place safely.

Amplite™ Fluorimetric Catalase Assay Kit (Cat# 11306) provides a quick and sensitive method for the measurement of catalase activity. Catalase reacts with H_2O_2 to produce water and oxygen. Amplite™ Red used in the assay reacts with H_2O_2 to generate a red fluorescent product. Therefore the reduction in fluorescence intensity is proportional to catalase activity. Amplite™ Red substrate enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With the Amplite™ Fluorimetric Catalase Assay Kit, we have detected as little as 30 mU/mL catalase in a 100 µL reaction volume.

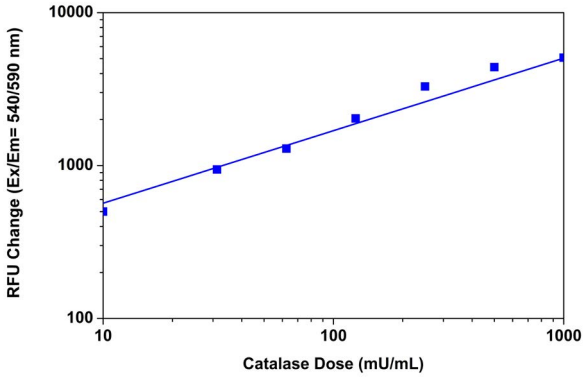

Figure 5.1. Catalase dose responses were measured with Amplite™ Fluorimetric Catalase Assay Kit (Cat# 11306) in a 96 well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 30 mU/mL catalase was detected with 30 minutes incubation time (n=3).

Table 5.1 Catalase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11306	Amplite™ Fluorimetric Catalase Assay Kit *Red Fluorescence*	200 tests	571	585
11501	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Red Fluorescence*	500 tests	575	590

5.2 Cytochrome P450

Metabolic oxidation of chemical compounds, including many pollutants, is the function of the cytochrome-mediated monooxygenase or mixed-function oxidase system. Cytochrome P450 (CYP) can be followed using various fluorogenic alkyl ether derivatives of coumarin, resorufin and fluorescein, all of which yield cleavage products with longer-wavelength spectral properties than the parent substrates.

Resorufin ether–based substrates, which all yield red-fluorescent resorufin with excitation/emission maxima ~571/585 nm, have been extensively used to differentiate isozymes of cytochrome P450. Fluorescence detection of the deethylation of 3-cyano-7-ethoxycoumarin is 50–100 times more sensitive than that of ethoxyresorufin, primarily because of the faster turnover rate of 3-cyano-7-ethoxycoumarin. The deethylase product of 3-cyano-7-ethoxycoumarin, 3-cyano-7-hydroxycoumarin has a lower pKa than that of 7-ethoxycoumarin, allowing continuous measurements of enzyme activity at pH 7. The cytochrome P450 substrate 7-ethoxy-4-trifluoromethylcoumarin yields a coumarin product with a fluorescence emission that is distinct from that of the substrate and of NADPH, making this substrate useful for the direct measurement of enzymatic activity. Researchers have shown that this substrate is cleaved by at least the 1A2, 2E1 and 2B1 isozymes of cytochrome P450.

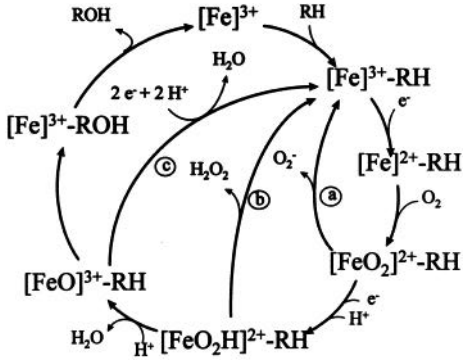

Figure 5.2. The catalytic cycle of cytochrome P450

Table 5.2 Cytochrome P450 Probes

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
15001	CEC [3-Cyano-7-ethoxycoumarin]	10 mg	408	450
15005	7-Ethoxy-4-trifluoromethylcoumarin	25 mg	385	502
15020	Resorufin Benzyl Ether	10 mg	571	585
15023	Resorufin Ethyl Ether	5 mg	571	585
15024	Resorufin Methyl Ether	5 mg	571	585
15025	Resorufin Pentyl Ether	5 mg	571	585

5.3 Glucose Oxidase

Glucose oxidase is a dimeric protein that catalyzes the oxidation of beta-D-glucose into hydrogen peroxide and D-glucono-1,5-lactone, which is hydrolyzed to gluconic acid. Glucose oxidase requires flavin adenine dinucleotide (FAD) as a cofactor. In the glucose oxidase-catalyzed redox reactions, FAD serves as the initial electron acceptor and is reduced to $FADH_2$. It is widely used for the determination of glucose in body fluids and in removing residual glucose and oxygen from beverages, food and other agricultural products. Furthermore, glucose oxidase is commonly used in biosensors to detect glucose.

Amplite™ Fluorimetric Glucose Oxidase Assay Kit (Cat# 11300) provides a quick and sensitive method for the measurement of glucose oxidase in solution. It can be performed in a convenient 96-well or 384-well microtiter-plate format and is easily adapted to automation without a separation step. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With Amplite™ Fluorimetric Glucose Oxidase Assay Kit, we have detected as little as 0.05 mU/mL glucose oxidase in a 100 µL reaction volume.

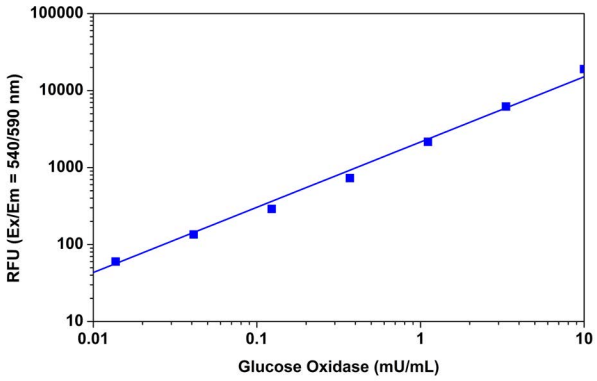

Figure 5.3. Glucose oxidase dose responses were measured with Amplite™ Glucose Oxidase Assay Kit (Cat# 11300) in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.05 mU/mL glucose oxidase was detected with 30 minutes incubation time (n=3).

5.4 Glutamate Oxidase

Glutamate oxidase belongs to the family of oxidoreductases, specifically those acting on the CH-NH_2 group of donors with oxygen as an acceptor. It is an enzyme that specifically catalyzes the oxidative deamination of L-glutamate in the presence of water and oxygen with the formation of α-ketoglutarate, ammonia, and hydrogen peroxide.

Amplite™ Fluorimetric Glutamate Oxidase Assay Kit (Cat# 11302) provides a quick and ultrasensitive method for the measurement of glutamate oxidase in solution and in cell lysates. In the assay, L-glutamic acid is oxidized to α-ketoglutarate, NH_3 and H_2O_2 by glutamate oxidase. L-Alanine and L-glutamate-pyruvate transaminase are included in the reaction, resulting in multiple cycles of the initial reaction, thus significantly amplifying the production of H_2O_2. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With Amplite™ Fluorimetric Glutamate Oxidase Assay kit, we have detected as little as 40 µU/mL glutamate oxidase in a 100 µL reaction volume.

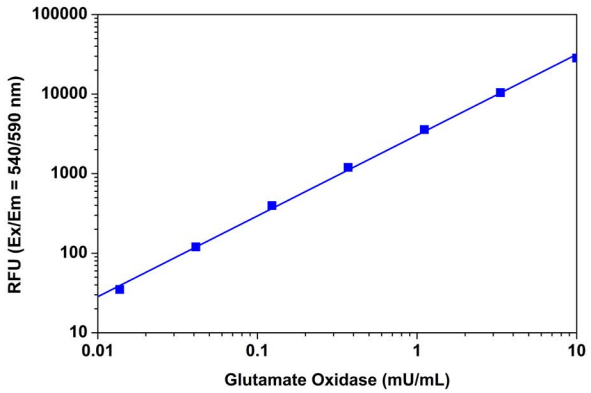

Figure 5.3. Glutamate oxidase dose responses were measured with Amplite™ Fluorescence Glutamate Oxidase Assay Kit (Cat# 11302) on a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 40 µU/mL glutamate oxidase was detected with 60 minutes incubation time (n=3).

Table 5.3 Glucose and Glutamate Oxidase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11300	Amplite™ Fluorimetric Glucose Oxidase Assay Kit *Red Fluorescence*	500 tests	571	585
11302	Amplite™ Fluorimetric Glutamate Oxidase Assay Kit *Red Fluorescence*	200 tests	571	585
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11501	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Red Fluorescence*	500 tests	575	590

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5.5 Horseradish Peroxidase

Horseradish Peroxidase (HRP) is a small molecule (MW ~40 KD) that is widely used in a variety of biological detections. HRP is a glycoprotein with 6 lysines which can be conjugated to a tag molecule (e.g., secondary antibodies and avidins). HRP conjugates are extensively used as secondary detection reagents in ELISAs, immuno-histochemical techniques as well as Northern, Southern and Western blot analyses. Due to its small size, HRP rarely causes steric hindrance problem with the antibody/antigen complex formation. It is usually conjugated to an antibody in a 4:1 ratio. Additionally, HRP is inexpensive compared to other labeling enzymes.

Amplite™ Colorimetric Peroxidase Assay Kit (Cat# 11551) uses Amplite™ Blue, our chromogenic HRP substrate that is much more sensitive to both H_2O_2 and peroxidase than other chromogenic peroxidase substrates such as TMB, ABTS, OPD and K-Blue. Amplite™ Blue generates a highly absorptive material that has maximum absorption of 664 nm. This near infrared absorption minimizes the background absorption often caused by the auto-absorption of biological samples. Its signal can be easily read by an absorbance microplate reader at ~664 nm.

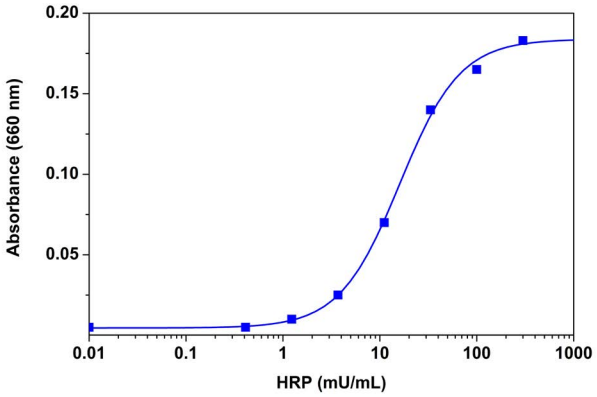

Figure 5.5. HRP dose responses were measured with Amplite™ Colorimetric Peroxidase Assay Kit (Cat# 11551) in a 96-well white wall/clear bottom plate. As low as 3 mU/mL of peroxidase was detected.

Amplite™ Fluorimetric Peroxidase Assay Kits are a one-step, homogeneous, no wash assay system. Kit 11553 uses fluorogenic Amplite™ IR substrate to quantify peroxidase in solutions. It can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screenings. The kit provides an optimized "mix and read" assay protocol. Kit 11553 can detect as low as 1 mU/mL of HRP. It can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 640/670 nm or an absorbance microplate reader at ~647 nm. Amplite™ IR might be the only commercially available fluorogenic HRP substrate. It can be potentially used in the *in vivo* imaging of small animals.

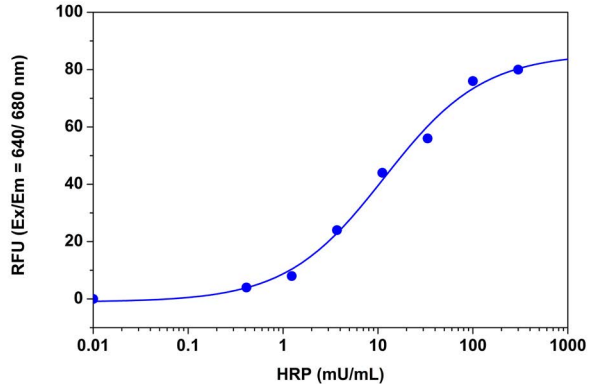

Figure 5.6. HRP dose responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11553) in a 384-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 1 mU/mL of peroxidase was detected with 30 minutes incubation (n=3).

Kit 11552 uses fluorogenic Amplite™ Red HRP substrate to quantify peroxidase in solutions. It can be used for ELISAs, characterizing kinetics of enzyme reaction, and high throughput screenings. The kit provides an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. It can detect as low as 10 µU/mL of HRP. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~576 nm.

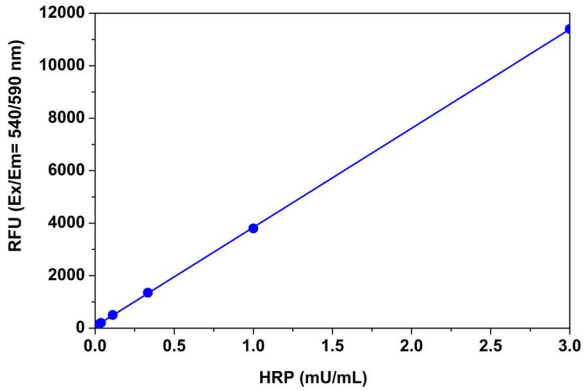

Figure 5.7. HRP dose responses were measured with Amplite™ Fluorimetric Peroxidase Assay Kit (Cat# 11552) in a 384-well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 10 µU/mL of peroxidase was detected with 30 minutes incubation (n=3).

Enhanced chemiluminescence is a common technique for a variety of detection assays in biology. A horseradish peroxidase enzyme (HRP) is tethered to the molecule of interest (usually through labeling an immunoglobulin that specifically recognizes the molecule). This enzyme complex catalyzes the conversion of the enhanced chemiluminescent substrate into a sensitized reagent in the vicinity of the molecule of interest. The further oxidation of the substrate by hydrogen peroxide produces an excited molecule which emits easily detectable light.

Amplite™ Luminometric Peroxidase Assay Kit (Cat# 11559) uses Amplite™ luminometric HRP substrate to quantify peroxidase in solutions. It provides an optimized "mix and read" assay protocol. The kit can detect as low as 100 µU/mL of HRP. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a luminescence microplate reader. The kit can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screenings.

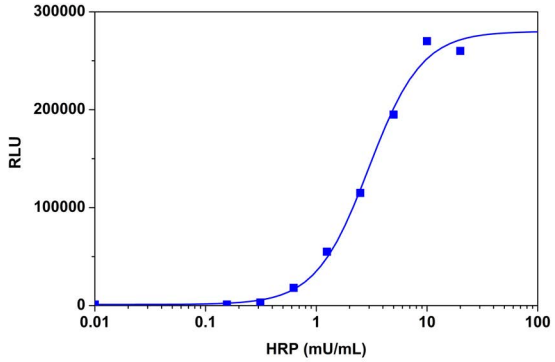

Figure 5.8. HRP dose responses were measured with Amplite™ Luminometric Peroxidase Assay Kit (Cat# 11559) in a 384 - well black plate. As low as 150 mU/mL of peroxidase can be detected with 30 minutes incubation time (n=3).

Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP conjugate ELISA Assay Kit (Cat# 11540) contains all the essential components including fluorogenic Amplite™ Red HRP substrate for ELISA detection. The kit provides an optimized assay protocol. It can detect as little as 0.4 ng/well of a monoclonal antibody. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~576 nm. It has been used for the assays in which goat anti-mouse IgG is served as a secondary detection agent.

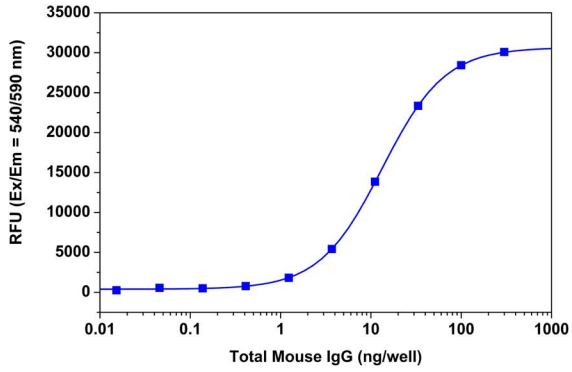

Figure 5.9. Detection of total mouse IgG using Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP Conjugate ELISA Kit (Cat# 11540). Mouse IgG was diluted into 3 µg/mL and made 1 to 3 serial dilutions in 0.2 M sodium bicarbonate buffer, pH 9.4. 100 µL/well serial dilutions were coated into a 96-well solid black plate at 4 °C overnight, and blocked with 3% milk in PBS and 0.02% Tween-20 at 4 °C overnight. The wells were washed, and assayed using the reagents. 1 to 5000 dilutions of goat anti-mouse IgG, HRP conjugate were used. The reactions were incubated for 10 to 60 minutes and then measured for fluorescence at Ex/Em = 540/590 nm using Gemini fluorescence microplate reader. As low as 0.4 ng/well of total mouse IgG was detected with 10 minutes incubation (n=3).

Kit 11541 can be used for the assays in which goat anti-rabbit IgG is served as the secondary detection agent. It provides an optimized assay protocol that is compatible with HTS liquid handling instruments. As little as 3 ng/well of a polyclonal antibody was detected. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~576 nm.

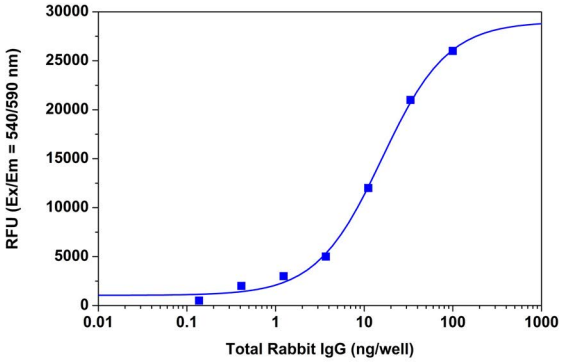

Figure 5.10. Detection of total rabbit IgG using Amplite™ Fluorimetric Goat Anti-Rabbit IgG-HRP Conjugate ELISA Assay Kit (Cat# 11541). Rabbit IgG was diluted into 1 µg/mL and made 1 to 3 serial dilutions in 0.2 M sodium bicarbonate buffer at pH 9.4. 100 µL/well serial dilutions were coated into a 96-well black plate at 4 °C overnight, and blocked with 3% milk in PBS and 0.02% Tween-20 at 4 °C overnight. 1 to 6000 dilutions of goat anti-rabbit IgG-HRP conjugate were used. As low as 3 ng/well of total rabbit IgG was detected with 30 minutes incubation (n=3).

Table 5.4 Horseradish Peroxidase Assay Probes and Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11000	ADHP	25 mg	571	585
11005	Amplite™ Blue	25 mg	324	409
11551	Amplite™ Colorimetric Peroxidase Assay Kit *Blue Color*	500 tests	664	N/A
11540	Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP Conjugate ELISA Assay Kit *Red Fluorescence*	10 plates	571	585
11541	Amplite™ Fluorimetric Goat Anti-Rabbit IgG-HRP Conjugate ELISA Assay Kit *Red Fluorescence*	10 plates	571	585
11553	Amplite™ Fluorimetric Peroxidase Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11552	Amplite™ Fluorimetric Peroxidase Assay Kit *Red Fluorescence*	500 tests	575	590
11559	Amplite™ Luminometric Peroxidase Assay Kit	500 tests	425	N/A
11011	Amplite™ Red HRP Substrate	1000 tests	571	585
11050	Luminol [3-Aminophthalhydrazide] *UltraPure grade*	1 g	355	411
11001	ReadiUse™ ABTS Solution *Optimized for ELISA Assays with HRP Conjugates*	1 L	420	N/A
11004	ReadiUse™ hydrogen peroxide solution *50 mM calibrated and stabilized solution*	5x10 mL	N/A	N/A
11010	Signal Guard™ HRP conjugate stabilizer	50 mL	N/A	N/A

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5.6 Lysyl Oxidase

Lysyl oxidase (LOX) is an extracellular enzyme that catalyzes formation of aldehydes from lysine residues in collagen and elastin precursors. These aldehydes are highly reactive, and undergo spontaneous chemical reactions with other lysyl oxidase-derived aldehyde residues or with unmodified lysine residues. The chemical reactions result in cross-linking collagen and elastin, which is essential for stabilization of collagen fibrils and for the integrity and elasticity of mature elastin. The activity of Lysyl oxidase in biological samples is traditionally assessed by tritium release end-point assays using radio isotope labeled collagen or elastin substrates. Amplite™ Fluorimetric Lysyl Oxidase Assay Kit (Cat# 15255) offers a sensitive fluorescent assay for detecting the activity of lysyl oxidase. It utilizes a proprietary LOX substrate that releases hydrogen peroxide detected using our Amplite™ ADHP substrate in HRP-coupled reactions. This method allows the detection of sub ng/mL lysyl oxidase and is much more sensitive than the currently available assays. It eliminates the interference that occurs in some biological samples and can be readily used to detect lysyl oxidase activity in cell extracts or solutions. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm.

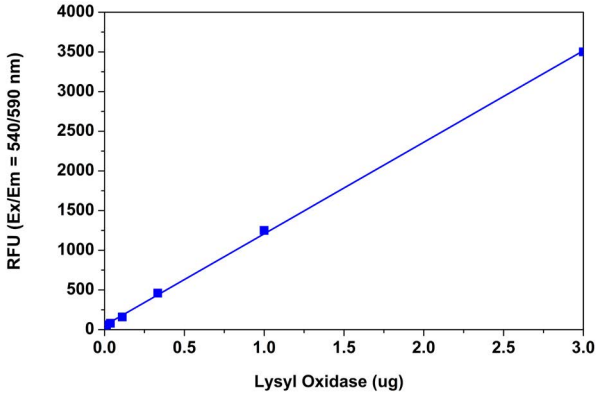

Figure 5.11. Lysyl oxidase dose responses were measured on a 96-well black solid plate with Amplite™ Fluorimetric Lysyl Oxidase Assay Kit (Cat# 15255). As low as 40 ng of lysyl oxidase was detected with 30 minutes incubation (n=3).

Table 5.5 Lysyl Oxidase Assay Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
15255	Amplite™ Fluorimetric Lysyl Oxidase Assay Kit *Red Fluorescence*	400 tests	571	585

Recent Citations:

1). F. Stoolzel etal. (2011). Blood, 118, 935

2). B. Copez etal. (2013), Cardiovasc. Res 99, 111.

3). C. P. El-Haibi (2012), PNAS, 109, 17460

4). E. Remus (2012), Am. J. Physiol. Heart Circ Physiol. 303, H1067

5.7 Monoamine Oxidase

Monoamine oxidases (MAOs) are a family of flavin-containing amine oxidoreductases that catalyze the oxidation of monoamines. They are found bound to the outer membrane of mitochondria in numerous tissues including liver, intestinal mucosa, and nerves. In humans, there are two types of MAO: MAO-A and MAO-B. MAO-A is particularly important in the metabolism of monoamines ingested in food. MAOs play a major role in the inactivation of neurotransmitters. MAO dysfunction has been associated with depression, schizophrenia, substance abuse, attention deficit disorder, migraines, and irregular sexual maturation.

Amplite™ Fluorimetric Monoamine Oxidase Assay Kit (Cat# 11303) provides a quick and sensitive method for the measurement of monoamine oxidase and semicarbazide-sensitive amine oxidase (SSAO) activities in blood samples and other biological samples. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With Amplite™ Fluorimetric Monoamine Oxidase Assay Kit, we have detected as little as 10 µU/mL SSAO in a 100 µL reaction volume. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

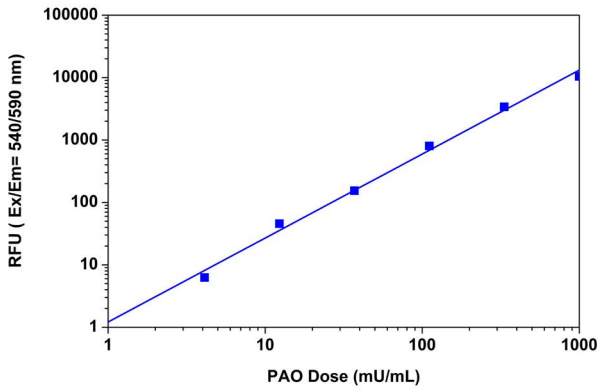

Figure 5.12. PAO dose responses were measured with Amplite™ Fluorimetric Monoamine Oxidase Assay Kit (Cat# 11303) in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 10 mU/mL PAO was detected with 30 minutes incubation (n=3).

Table 5.6 Monoamine Oxidase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11501	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Red Fluorescence*	500 tests	575	590
11303	Amplite™ Fluorimetric Monoamine Oxidase Assay Kit *Red Fluorescence*	200 tests	571	585

5.8 Myeloperoxidase

Myeloperoxidase (MPO), most abundantly present in neutrophils and monocytes, is a green hemoprotein having peroxidase activity. MPO is a 150 kD dimerprotein consisting of two 15 kD light chains and two variable weight glycosylated heavy chains bound to a prosthetic heme group. It catalyzes the reaction of hydrogen peroxide and halide ions to form cytotoxic acids and other intermediates; and plays an important role in the oxygen-dependent killing of tumor cells and microorganisms. MPO deficiency is a hereditary deficiency of the enzyme, which predisposes to immune deficiency. There are considerable interests in the development of therapeutic MPO inhibitors.

Amplite™ Fluorimetric Myeloperoxidase Assay Kit (Cat# 11301) provides a quick and sensitive method for the measurement of myeloperoxidase in solution and in cell lysates. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With Amplite™ Fluorimetric Myeloperoxidase Assay Kit, we have detected as little as 5 ng/mL myeloperoxidase in a 100 µL reaction volume. The kit can be automated for high throughput screenings of MPO inhibitors.

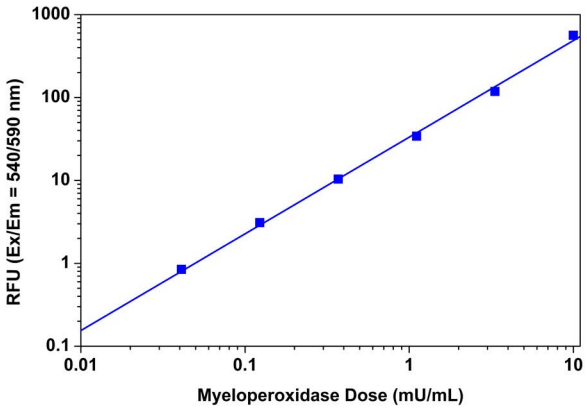

Figure 5.13. Myeloperoxidase dose responses were measured with Amplite™ Fluorimetric Myeloperoxidase Assay Kit (Cat# 11301) in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.1 mU/mL myeloperoxidase was detected with 60 minutes incubation (n=3).

5.9 Superoxide Dismutase

Superoxide dismutases (SODs) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide is one of the main reactive oxygen species in cells. It is a substantial contributor of pathology associated with neurodegenerative diseases, ischemia reperfusion injury, atherosclerosis and aging. SODs are an important antioxidant defense in nearly all cells exposed to superoxide radicals. In fact, mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma, an acceleration of age-related muscle mass loss, an earlier incidence of cataracts and a reduced lifespan. Overexpression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation.

Amplite™ Colorimetric Superoxide Dismutase (SOD) Assay Kit (Cat# 11305) provides a quick and sensitive method for the measurement of SOD activity in solutions. In the assay, xanthine is converted to superoxide radical ions, uric acid and hydrogen peroxide by xanthine oxidase (XO). Superoxide reacts with SOD Orange™ to generate a product that absorbs around 560 nm. SOD inhibits the reaction of SOD Orange™ with superoxide, thus reduces the absorption at 560 nm. The reduction in the absorption of SOD Orange™ at 560 nm is proportional to SOD activity. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format.

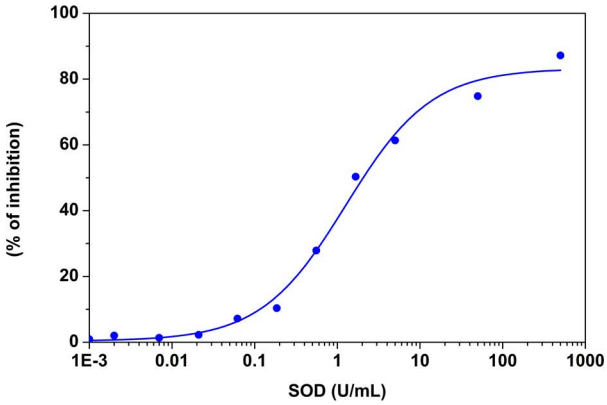

Figure 5.14. SOD dose responses were measured with Amplite™ Colorimetric Superoxide Dismutase (SOD) Assay Kit (Cat# 11305) in a 96-well white wall/clear bottom plate with a Spectrum Max microplate reader (Molecular Devices). As low as 0.1 U/mL SOD was detected with 60 minutes incubation (n=3). .

Table 5.7 Myeloperoxidase and Superoxide Dismutase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11501	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Red Fluorescence*	500 tests	575	590
11301	Amplite™ Fluorimetric Myeloperoxidase Assay Kit *Red Fluorescence*	200 tests	571	585
11305	Amplite™ Colorimetric Superoxide Dismutase (SOD) Assay Kit	200 tests	560	N/A

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5.10 Xanthine Oxidase

Xanthine oxidase (XO) is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. It plays an important role in the catabolism of purines. Xanthine oxidase is normally found in liver and jejunum. During severe liver damage, xanthine oxidase is released into blood, so a blood assay for XO is a way to determine if liver damage has happened. Xanthinuria is a rare genetic disorder where the lack of xanthine oxidase leads to high concentration of xanthine in blood and can cause health problems such as renal failure.

Amplite™ Fluorimetric Xanthine Oxidase Assay Kit (Cat# 11304) provides a quick and ultrasensitive method for the measurement of xanthine oxidase activities. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. In the assay, xanthine oxidase catalyzes the oxidation of purine bases, hypoxanthine or xanthine to uric acid and superoxide , which spontaneously degrades to hydrogen peroxide (H_2O_2). The kit uses Amplite™ Red substrate which enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With Amplite™ Fluorimetric Xanthine Oxidase Assay Kit, we have detected as little as 0.15 mU/mL xanthine oxidase in a 100 µL reaction volume.

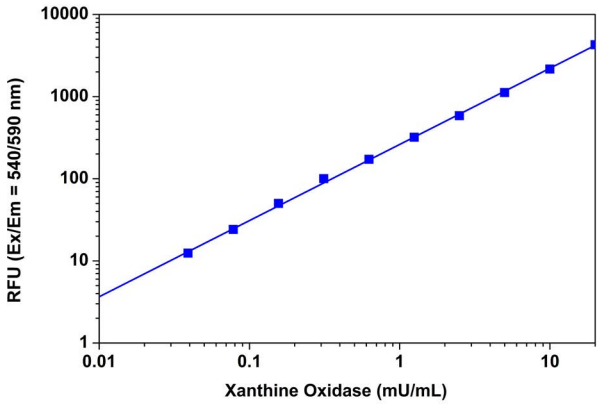

Figure 5.15. Xanthine oxidase dose responses were measured with Amplite™ Fluorimetric Xanthine Oxidase Assay Kit (Cat# 11304) on a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.15 mU/mL xanthine oxidase was detected with 30 minutes incubation time (n=3).

Table 5.10 Xanthine Oxidase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11501	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Red Fluorescence*	500 tests	575	590
11304	Amplite™ Fluorimetric Xanthine Oxidase Assay Kit *Red Fluorescence*	200 tests	575	590

5.11 Other Oxidases

Oxidases are a subclass of the oxidoreductases. An oxidase is any enzyme that catalyzes an oxidation-reduction reaction involving molecular oxygen as the electron acceptor. In microbiology, the oxidase test is used as a phenotypic characteristic for the identification of bacterial strains. Oxidase test determines whether a given bacterium produces cytochrome oxidases (and therefore utilizes oxygen with an electron transfer chain). In oxidase reactions, oxygen is reduced to water (H_2O) or hydrogen peroxide (H_2O_2). The production of hydrogen peroxide can be conveniently used for monitoring and detecting oxidase activities.

Hydrogen peroxide (H_2O_2) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states. It is involved in a number of biological events that have been linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, neurodegenerative diseases and Down's syndrome. Measurement of this reactive species will help to determine how oxidative stress modulates a variety of intracellular pathways.

Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit 11502 uses our unique Amplite™ IR peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. Amplite™ IR generates the fluorescence that is pH-independent from pH 4 to 10. It is a superior alternative to ADHP (Amplex® Red) for the detections that require low pH where ADHP has reduced fluorescence. In addition, Amplite™ IR generates a product that has maximum absorption at 647 nm with maximum emission at 670 nm. This near infrared fluorescence minimizes the assay background that is often caused by the autofluorescence of biological samples. It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. Kit 11502 provides a sensitive, one-step fluorometric assay to detect as little as 3 picomoles of H_2O_2 in a 100 µL assay volume (30 nM). The assay can be performed in a convenient 96-well or 384-well microplate, and easily adapted to automation without a separation step.

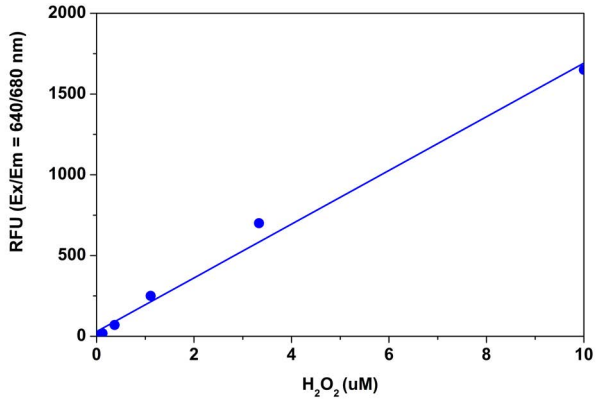

Figure 5.16. H_2O_2 dose responses were measured in a 96-well black solid plate with the Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502). As low as 0.03 µM H_2O_2 was detected.

Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit 11501 uses our non-fluorescent Amplite™ Red peroxidase substrate to quantify hydrogen peroxide in solutions and cell extracts. It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. Kit 11501 is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments. It provides a sensitive, one-step fluorometric assay to detect as little as 3 pico-moles of H_2O_2 in a 100 µL assay volume (30 nM). Its signal can be easily read using either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~570 nm.

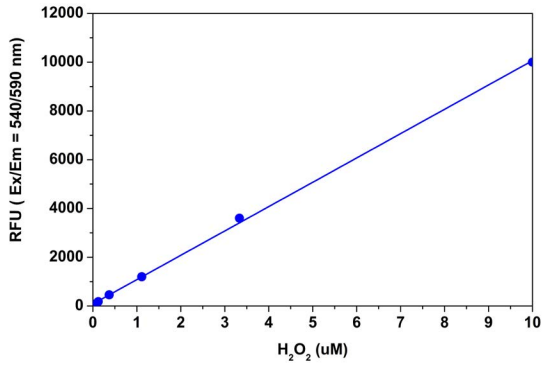

Figure 5.17. H_2O_2 dose responses were measured in a 384-well black solid plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11501). As low as 0.03 µM H_2O_2 was detected with 30 minutes incubation (n=3).

Very reactive aldehydes, namely 4-hydroxyalkenals, were first shown to be formed in autoxidizing chemical systems. It was subsequently shown that 4-hydroxyalkenals, particularly 4-hydroxynonenal, were formed in substantial amounts under biological conditions, i.e. during the peroxidation of lipids of liver microsomes incubated in the NADPH-Fe system. Many other aldehydes were also identified in peroxidizing liver microsomes or hepatocytes, e.g., alkanals , alk-2-enals, and 4-hydroxyalkenals. The formation, reactivity and toxicity of aldehydes originating from the peroxidation of lipids of cellular membranes have received great attention in recent years. Rapid and accurate measurement of aldehydes is an important task for biological research, chemical research, food industry and environmental pollution surveillance. There are few reagents or assay kits available for quantifying the number of aldehydes. Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS.

AAT Bioquest's aldehyde detection kits might be used for monitoring the oxidases that use an aliphatic amine as a substrate to generate an aldehyde as its oxidation product. For example, monoamine oxidase uses benzylamine as a substrate to produce benzaldehyde, which can be monitored by using our aldehyde detection kits. Amplite™ Colorimetric Aldehyde Quantitation kit (Cat# 10051) uses a proprietary dye that generates a chromogenic product upon reacting with an aldehyde. The kit provides a sensitive, one-step colorimetric method to detect as little as 1 nanomole of aldehyde in a 100 µL assay volume (10 µM). Its signal can be easily read with an absorbance microplate reader at 405 or 550 nm.

Both Amplite™ Colorimetric Aldehyde Qutitation Kit (Cat# 10051) and Amplite™ Fluorimetric Aldehyde Qutitation kit (Cat# 10052) are used for quantifying aldehydes at higher pH. Kit 10052 uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with an aldehyde. Kit 10052 is much more sensitive than Kit 10051. It provides a mix-and-read method to detect as little as 0.1 nanomole of aldehyde in a 100 µL assay volume (1 µM). Its signal can be easily read using a fluorescence microplate reader at Ex/Em = 365/435 nm.

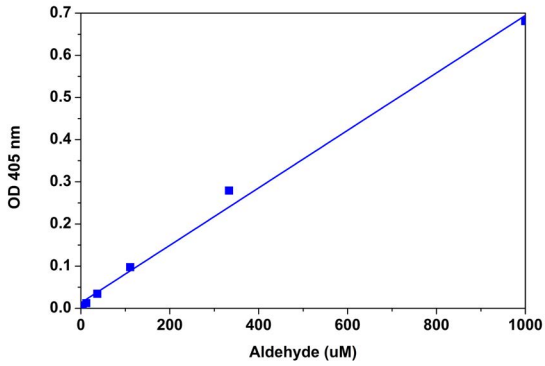

Figure 5.18. Aldehyde dose responses were measured in a 96-well clear plate with Amplite™ Colorimetric Aldehyde Quantitation Assay Kit (Cat# 10051). As low as 10 µM (1 nanomol/well) of aldehyde was detected.

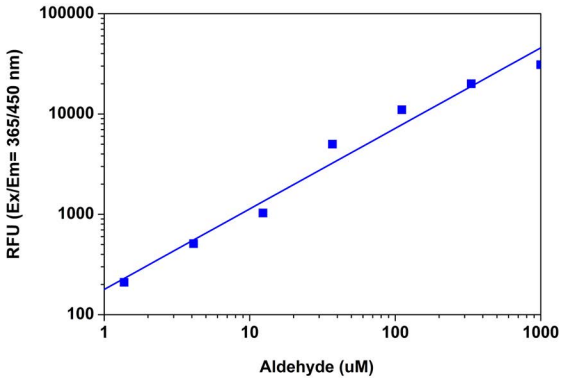

Figure 5.19. Aldehyde dose responses were measured in a solid black 96-well plate with Amplite™ Fluorimetric Aldehyde Quantitation Kit (Cat# 10052). As low as 3 µM of aldehyde was detected with 15 minutes incubation (n=3).

Table 5.11 Oxidase Detection Reagents and Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
11000	Amplite™ ADHP [10-Acetyl-3,7-dihydroxyphenoxazine]	25 mg	571	585
11005	Amplite™ Blue	25 mg	324	409
10051	Amplite™ Colorimetric Aldehyde Quantitation Kit	200 tests	550	N/A
10053	Amplite™ Colorimetric Aldehyde Quantitation Kit *Blue Color*	200 tests	620	N/A
10052	Amplite™ Fluorimetric Aldehyde Quantitation Kit *Blue Fluorescence*	200 tests	360	450
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11501	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Red Fluorescence*	500 tests	575	590
11009	Amplite™ IR	1 mg	647	670
11011	Amplite™ Red HRP Substrate	1000 tests	571	585

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Dehydrogenases 6

dehydrogenase probes and assay kits at-a-glance*

Enzyme	Colorimetric	Fluorimetric
Glucose-6-Phosphate Dehydrogenase	13807	13805
Lactate Dehydrogenase (LDH)	13809, 13813	13808, 13812
NAD/NADH	15258, 15271, 15273, 15275	15257, 15261, 15263
NADP/NADPH	15260, 15272, 15274, 15276	15257, 15259, 15262, 15264,

* products listed by catalog number

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Dehydrogenases

6.1 Glucose-6-Phosphate Dehydrogenase

Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone, the first and rate-limiting step in the pentose phosphate pathway. It is critical metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH), and for the production of pentose sugars. The production of NADPH is of great importance for tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. NADPH also maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage. Deficiencies in G6PD predispose individuals to non-immune hemolytic anemia.

AAT Bioquest Amplite™ Fluorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit (Cat# 13806) provides a simple, sensitive and rapid fluorescence-based method for detecting G6PD in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, G6PD activity is proportionally related to the concentration of NADPH that is specifically monitored using a fluorogenic NADPH sensor to yield a highly red fluorescence product. The fluorescence signal can be read with a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. With the G6PD assay kit, we were able to detect as little as 0.3 mU/mL G6PD in a 100 µL reaction volume. It is robust, and can be readily adapted for a wide variety of applications that require the measurement of G6PD.

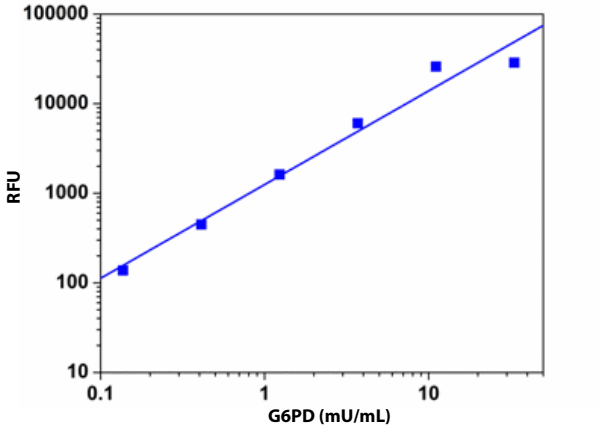

Figure 6.1. G6PD dose responses were measured with Amplite™ Fluorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit (Cat# 13806) in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 0.3 mU/mL glucose-6-phosphate dehydrogenase in 100 µL volume can be detected with 1 hour incubation.

Amplite™ Colorimetric Glucose-6-Phosphate Dehydrogenase Assay Kit (Cat# 13807) provides a simple, sensitive and rapid absorbance-based method for detecting G6PD in biological samples such as serum, plasma, urine, as well as in cell culture samples. The absorption signal can be read with an absorption microplate reader at ~575 nm or at the absorbance ratio of A_{575}nm/A_{605}nm to increase assay sensitivity. With the G6PD assay kit, we were able to detect as little as 3 mU/mL G6PD in a 100 µL reaction volume. It is robust, and can be readily adapted for a wide variety of applications that require the measurement of G6PD.

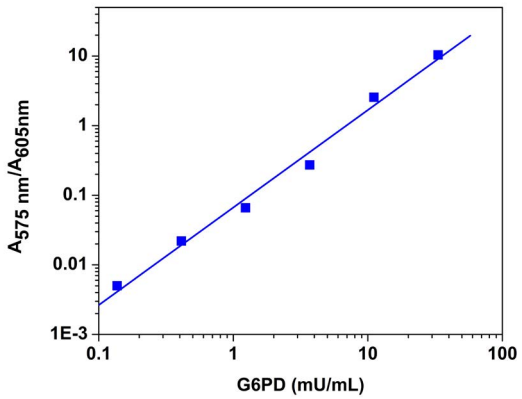

Figure 6.2. G6PD dose responses were measured with Amplite™ Colorimetric Glucose-6-phosphate dehydrogenase Assay Kit (Cat# 13807) in a white clear bottom plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 3 mU/mL glucose-6-phosphate dehydrogenase in 100 µL volume can be detected with 1 hour incubation.

Table 6.1 Glucose-6-Phosphate Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13805	Amplite™ Colorimetric Glucose-6-Phosphate Assay Kit	200 tests	575	N/A
13807	Amplite™ Colorimetric Glucose-6-Phosphate Dehydrogenase (G6PD) Assay Kit	200 tests	575	N/A
13804	Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit	200 tests	571	585
13806	Amplite™ Fluorimetric Glucose-6-Phosphate Dehydrogenase (G6PD) Assay Kit	200 tests	571	585

6.2 Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyzes the interconversion of pyruvate and lactate. Localized in the cytosol, LDH is present in a wide variety of organisms, including animals and plants. Cells release LDH into the bloodstream after tissue damage or red blood cell hemolysis. Since LDH is a fairly stable enzyme, it has been extensively used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has a broad range of applications.

AAT Bioquest Amplite™ Lactate Dehydrogenase Assay Kits provide

both fluorescence- and absorbance-based methods for detecting either L-lactate dehydrogenase (L-LDH) or D-lactate dehydrogenase (D-LDH). The assays are robust, and can be readily adapted for a wide variety of applications that require the measurement of L-LDH/D-LDH.

Amplite™ Lactate Dehydrogenase Assay Kits 13809 and 13813 provide an absorption-based method for detecting lactate dehydrogenases in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, LDH is proportionally related to the concentration of NADH that is specifically monitored by a chromogenic NADH sensor. The assays are specific for LDH. The absorption signal can be read using an absorption microplate reader at ~575 nm. With these LDH assay kits, we were able to detect as little as 3 mU/mL lactate dehydrogenase in a 100 µL reaction volume.

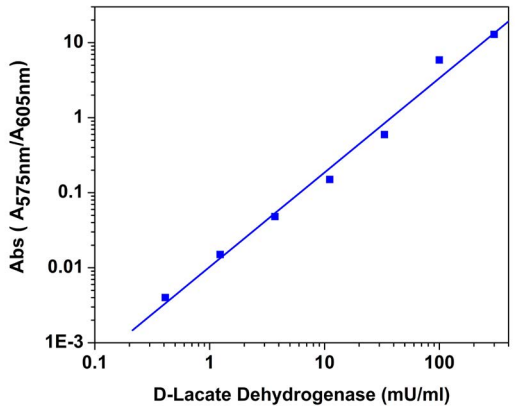

Figure 6.3. D-LDH dose responses were measured with Amplite™ Colorimetric D-LDH Assay Kit (Cat# 13809) in a 96-well black plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 3 mU/mL D-LDH in 100 µL volume was detected with 30 minutes incubation.

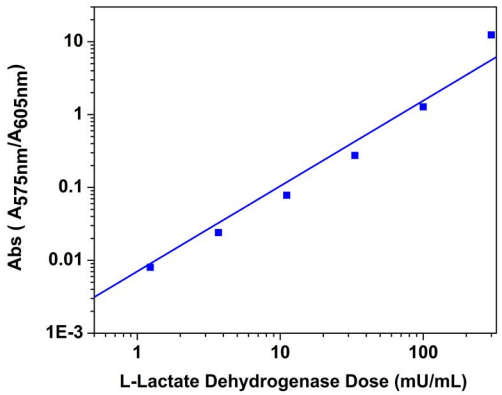

Figure 6.4. L-LDH dose responses were measured with Amplite™ Colorimetric L-Lactate Dehydrogenase Assay Kit (Cat# 13813) in a 96-well black plate using a SpectraMax Plus (Molecular Devices) microplate reader. As low as 3 mU/mL L-LDH in 100 µL volume was detected with 30 minutes incubation.

Amplite™ Lactate Dehydrogenase Assay Kits 13808 and 13812 provide a fluorescence-based method for detecting lactate dehydrogenase. In the enzyme coupled assays, LDH is proportionally related to the concentration of NADH that is specifically monitored by a fluorogenic NADH sensor. The assays are specific for LDH. The fluorescence signal can be read using a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. With these LDH assay kits, we were able to detect as little as 1 mU/mL lactate dehydrogenase in a 100 µL reaction volume.

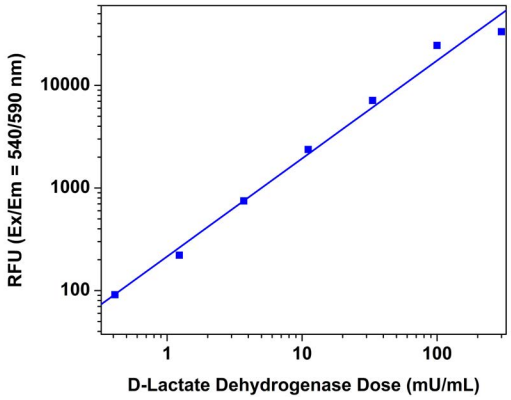

Figure 6.5. D-LDH dose responses were measured with Amplite™ Fluorimetric D-LDH Assay Kit (Cat# 13808) in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 1 mU/mL D-LDH in a 100 µL volume was detected with 30 minutes incubation.

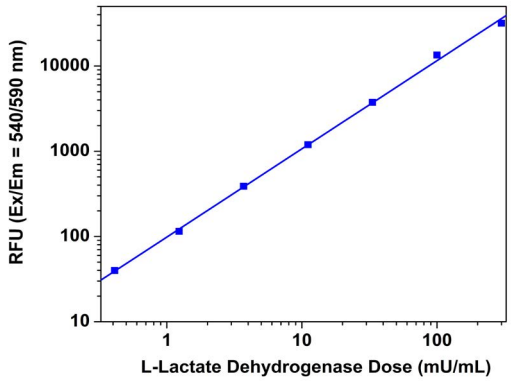

Figure 6.6. L-LDH dose responses were measured with Amplite™ Fluorimetric L-Lactate Dehydrogenase Assay Kit (Cat# 13812) in a 96-well black plate using a Gemini (Molecular Devices) microplate reader. As low as 1 mU/mL L-LDH in a 100 µL volume was detected with 30 minutes incubation.

Table 6.2 Lactate Dehydrogenase Assay Kits

Cat. #	Product Name	Size
13811	Amplite™ Colorimetric D-Lactate Assay Kit	200 tests
13809	Amplite™ Colorimetric D-Lactate Dehydrogenase (LDH) Assay Kit	200 tests
13815	Amplite™ Colorimetric L-Lactate Assay Kit	200 tests
13813	Amplite™ Colorimetric L-Lactate Dehydrogenase (LDH) Assay Kit	200 tests
13810	Amplite™ Fluorimetric D-Lactate Assay Kit	200 tests
13808	Amplite™ Fluorimetric D-Lactate Dehydrogenase (LDH) Assay Kit	200 tests
13814	Amplite™ Fluorimetric L-Lactate Assay Kit	200 tests
13812	Amplite™ Fluorimetric L-Lactate Dehydrogenase (LDH) Assay Kit	200 tests

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6.3 Other Dehydrogenases

A dehydrogenase is an enzyme that oxidizes a substrate by a reduction reaction that transfers one or more hydrides to an electron acceptor or coenzyme, usually nicotinamide adenine dinucleotide (NAD+ or NADH), nicotinamide adenine dinucleotide phosphate (NADP+ or NADPH), flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN). There are a number of dehydrogenases that have been identified, such as:

- *aldehyde dehydrogenase*
- *acetaldehyde dehydrogenase*
- *alcohol dehydrogenase*
- *glutamate dehydrogenase*
- *lactate dehydrogenase*
- *pyruvate dehydrogenase*
- *glucose-6-phosphate dehydrogenase*
- *glyceraldehyde-3-phosphate dehydrogenase*
- *sorbitol dehydrogenase*
- *isocitrate dehydrogenase*
- *alpha-ketoglutarate dehydrogenase*
- *succinate dehydrogenase*
- *malate dehydrogenase*

The change of NAD/NADH and NADP/NADPH can be readily used for monitoring a dehydrogenase that uses NAD/NADH or NADP/NADPH as an cofactor. The traditional NAD/NADH and NADP/NADPH assays are done by monitoring the changes in NADH or NADPH absorption at 340 nm. The short UV wavelength of the traditional NAD/NADH and NADP/NADPH assays makes these methods to suffer low sensitivity and high interference. Due to the weak absorption of NAD and NADH, the UV absorption method requires large sample sizes, making the same NAD and NADH measurement unpractical if the availability of samples is limited. AAT Bioquest has developed a full product line for detecting NAD, NADH, NADP and NADPH either spectrophotometrically or fluorimetrically.

Amplite™ NAD/NADH Assay Kits provides a convenient method for sensitive detection of NAD and NADH. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction that significantly increases detection sensitivity. In addition, the assays have very low background since they are performed in the red visible range that considerably reduces the interference resulted from biological samples. There is also no need to purify NAD/NADH from sample mix. Besides the fluorimetric assay kits, we offer 6 colorimetric assay kits for detecting NAD, NADH, NADP, and NADPH. These kits use longer wavelengths (585 nm or 635 nm) as the detection window. These longer absorption significantly reduce the interferences from other absorbing species that generally absorbs light in the UV and short visible wavelengths.

Amplite™ NADP/NADPH Assay Kits provide a convenient method for sensitive detection of NADP and NADPH. There is no need to purify NADP/NADPH from sample mix. Amplite™ Fluorimetric Total NADP/NADPH Assay Kit (Cat# 15259) or Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit (Cat# 15264) is recommended if higher sensitivity is required. The fluorescence signal can be recorded at Ex/Em = 540/590 nm.

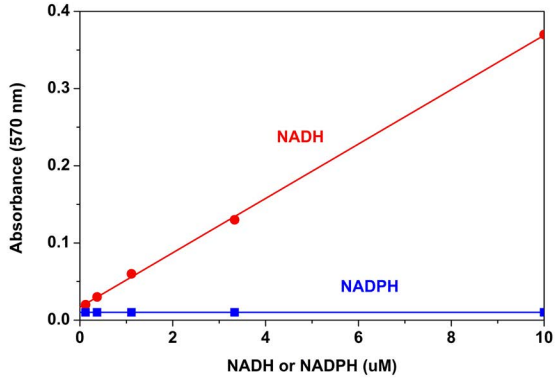

Figure 6.7. NADH dose responses were measured with Amplite™ Colorimetric NAD/NADH Assay Kit (Cat# 15258) in a 96-well white wall/clear bottom plate using a NOVOStar (BMG Labtech) microplate reader. As low as 300 nM (30 pmol/well) of NADH can be detected with 1 hour incubation time (n=3) while there is no response from NADPH.

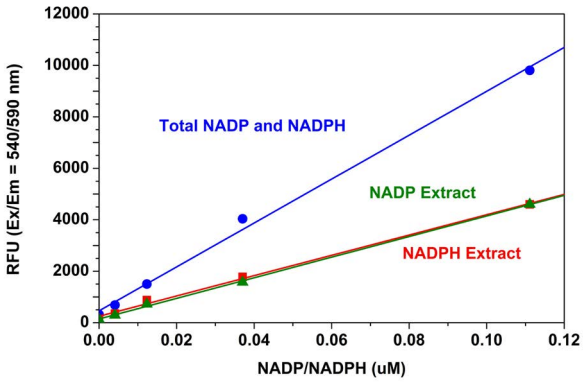

Figure 6.8. Total NADPH and NADP, and their extract dose responses were measured with Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit (Cat# 15264) in a 96-well black plate using a Gemini microplate reader (Molecular Devices). The signals were acquired at Ex/Em = 540/590 nm (cut off at 570 nm) 30 minutes after adding 75 µL of NADPH reaction mixture.

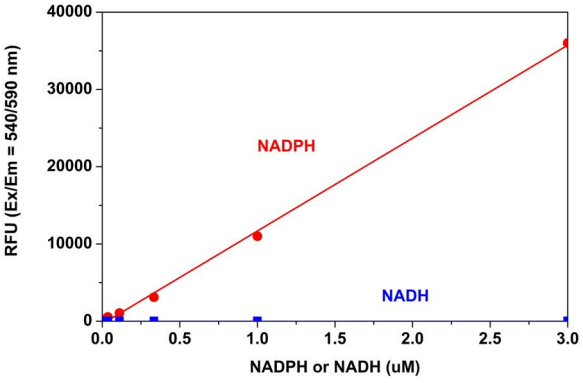

Figure 6.9. NADPH dose responses were measured with Amplite™ Fluorimetric Total NADP/NADPH Assay Kit (Cat# 15259) in a 96-well black solid plate. As low as 10 nM (1 pmol/well) of NADPH was detected with 30 minutes incubation time (n=3) while there was no response from NADH.

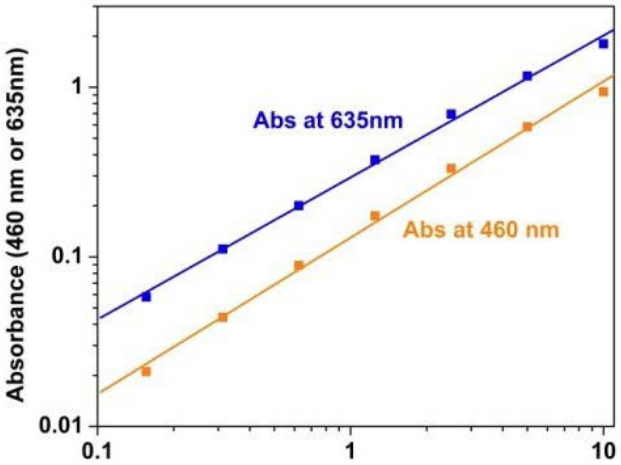

Figure 6.10. NADH dose responses were measured with Amplite™ Colorimetric Total NAD and NADH Assay Kit (Cat# 15275) in a 96-well white/clear bottom plate using a SpectraMax microplate reader (Molecular devices). As low as 0.1 µM of NADH can be detected with 1 hour incubation (n=3). Orange: Absorbance measurement at 460 nm, Blue: Absorbance measurement at 635 nm after adding enhancer to each well.

Table 6.3 NAD, NADH, NADP and NADPH Assay Kits

Cat. #	Product Name	Size
15273	Amplite™ Colorimetric NAD/NADH Ratio Assay Kit	400 tests
15271	Amplite™ Colorimetric NADH Assay Kit	400 tests
15274	Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit	400 tests
15272	Amplite™ Colorimetric NADPH Assay Kit	400 tests
15258	Amplite™ Colorimetric Total NAD and NADH Assay Kit	400 tests
15275	Amplite™ Colorimetric Total NAD and NADH Assay Kit *Enhanced Sensitivity*	400 tests
15260	Amplite™ Colorimetric Total NADP and NADPH Assay Kit	400 tests
15276	Amplite™ Colorimetric Total NADP and NADPH Assay Kit *Enhanced Sensitivity*	400 tests
15270	Amplite™ Fluorimetric Coenzyme A Quantitation Kit *Green Fluorescence*	200 tests
15263	Amplite™ Fluorimetric NAD/NADH Ratio Assay Kit *Red Fluorescence*	250 tests
15261	Amplite™ Fluorimetric NADH Assay Kit *Red Fluorescence*	400 tests
15264	Amplite™ Fluorimetric NADP/NADPH Ratio Assay Kit *Red Fluorescence*	250 tests
15262	Amplite™ Fluorimetric NADPH Assay Kit *Red Fluorescence*	400 tests
15257	Amplite™ Fluorimetric Total NAD and NADH Assay Kit *Red Fluorescence*	400 tests
15259	Amplite™ Fluorimetric Total NADP and NADPH Assay Kit *Red Fluorescence*	400 tests
15266	ReadiUse™ NADP Regenerating Kit	400 tests
15265	ReadiUse™ NADPH Regenerating Kit	1000 tests

Recent Citations of NAD, NADH, NADP and NADPH Assay Kits

Chao Tong, Alex Morrison, Samantha Mattison, Su Qian, Mark Bryniarski, Bethany Rankin, Jun Wang, D. Paul Thomas, and Ji Li. Impaired SIRT1 nucleocytoplasmic shuttling in the senescent heart during ischemic stress. FASEB J, Nov 2013; 27: 4332 - 4342.

Rubin Tan, Jiansha Li, Xiaochun Peng, Liping Zhu, Lei Cai, Tao Wang, Yuan Su, Kaikobad Irani, and Qinghua Hu. GAPDH is critical for superior efficacy of female bone marrow-derived mesenchymal stem cells on pulmonary hypertension. Cardiovasc Res, Oct 2013; 100: 19 - 27.

Kate J. Roberts, Andrew Cross, Olga Vasieva, Robert J. Moots, and Steven W. Edwards. Inhibition of pre-B cell colony-enhancing factor (PBEF/NAMPT/visfatin) decreases the ability of human neutrophils to generate reactive oxidants but does not impair bacterial killing. J. Leukoc. Biol., Sep 2013; 94: 481 - 492.

Stephen Y. Xue, Valeria Y. Hebert, Danicia M. Hayes, Corie N. Robinson, Mitzi Glover, and Tammy R. Dugas. Nucleoside Reverse Transcriptase Inhibitors Induce a Mitophagy-Associated Endothelial Cytotoxicity That Is Reversed by Coenzyme Q10 Cotreatment. Toxicol. Sci., Aug 2013; 134: 323 – 334.

Weijing Cai, Maya Ramdas, Li Zhu, Xue Chen, Gary E. Striker, and Helen Vlassara. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. PNAS, Sep 2012; 109: 15888 - 15893.

Yue Qiu, Claus Tittiger, Claude Wicker-Thomas, Gaëlle Le Goff, Sharon Young, Eric Wajnberg, Thierry Fricaux, Nathalie Taquet, Gary J. Blomquist, and René Feyereisen. An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis. PNAS, Sep 2012; 109: 14858 - 14863.

Nathalie Taquet, Gary J. Blomquist, and René Feyereisen. An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis. PNAS, Sep 2012; 109: 14858 - 14863.Yue Qiu, Claus Tittiger, Claude Wicker-Thomas, Gaëlle Le Goff, Sharon Young, Eric Wajnberg, Thierry Fricaux.

Jaime Uribarri, Weijing Cai, Maya Ramdas, Susan Goodman, Renata Pyzik, Xue Chen, Li Zhu, Gary E. Striker, and Helen Vlassara. Restriction of Advanced Glycation End Products Improves Insulin Resistance in Human Type 2 Diabetes: Potential role of AGER1 and SIRT1. Diabetes Care 2011; 34: 1610 - 1616

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Polymerases 7

Polymerases

A polymerase is an enzyme (EC 2.7.7.6/7/19/48/49) whose central biological function is the synthesis of polymers of nucleic acids. DNA polymerase and RNA polymerase are used to assemble DNA and RNA molecules, respectively, generally by copying a DNA or RNA template strand using base-pairing interactions. One particular polymerase, from the thermophilic bacterium, Thermus aquaticus (Taq) (PDB 1BGX, EC 2.7.7.7) is of vital commercial importance due to its use in the polymerase chain reaction (PCR), a widely used technique of molecular biology. Other well-known polymerases include terminal deoxynucleotidyl transferase (TDT) and reverse transcriptase, an enzyme used by RNA retroviruses like HIV. Reverse transcriptase is used to create a complementary strand to the preexisting strand of viral RNA before it can be integrated into the DNA of the host cell. It is a major target for antiviral drugs.

Polymerases use nucleotide triphosphates as substrates to produce pyrophosphate (PPi) as a product, thus the detection of PPi formation becomes a convenient tool for monitoring polymerase activities. Pyrophosphate (PPi) are produced by a number of biochemical reactions, such as ATP hydrolysis, DNA and RNA polymerizations, cyclic AMP formation by the enzyme adenylate cyclase and the enzymatic activation of fatty acids to form their coenzyme A esters.

PhosphoWroks™ Fluorimetric Pyrophosphate Assay Kits (Cat# 21611 and 21614) provide the most robust spectrophotometric method for the measurement of pyrophosphate. Both kits use our proprietary fluorogenic pyrophosphate sensors that have their fluorescence intensity proportionally dependent upon the concentration of pyrophosphate. The assays are much easier and more robust than enzyme-coupling pyrophosphate methods, which require at least two enzymes for their pyrophosphate detections. Due to their direct measurement of pyrophosphate, kit 21611 and 21614 are ideal for screening inhibition or activities of enzymes that consume or generate pyrophosphate. The assays can be performed in a convenient 96-well or 384-well microtiter-plate format. They are optimized mix-and-read assays. The kits provide all the essential components for assaying pyrophosphate.

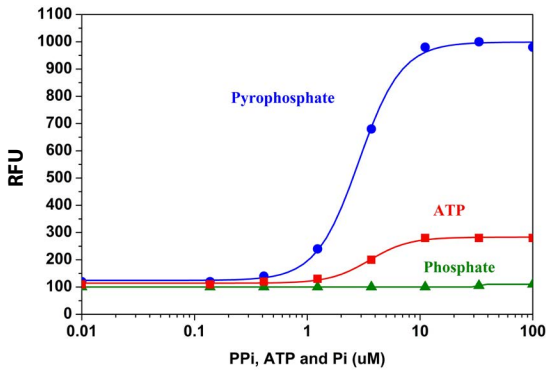

Figure 7.1. Pyrophosphate and phosphate dose responses were measured with PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit (Cat# 21611) in a 96-well black solid plate. As low as 1 µM (100 picmoles/well) pyrophosphate can be detected with 10 minutes incubation time.

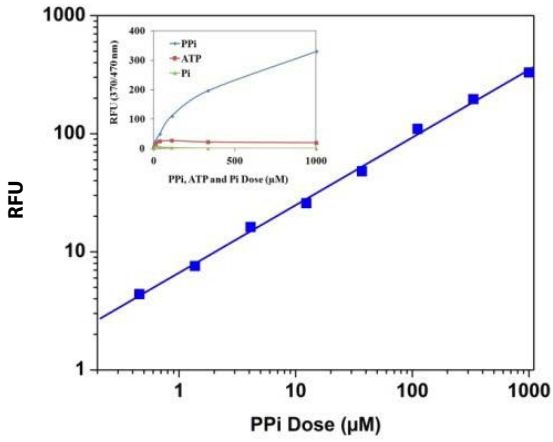

Figure 7.2. Pyrophosphate, ATP and phosphate dose responses were measured with PhosphoWorks™ Fluoremetric Pyrophosphate Assay Kit (Cat# 21614) in a solid black 96-well plate using a fluorescence microplate reader. As low as 1 µM (100 picmoles/well) pyrophosphate was detected with 10 minutes incubation.

Table 7.1 Polymerase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
21655	PhosphoWorks™ Fluorimetric ADP Assay Kit *Red Fluorescence*	100 tests	575	590
21611	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Blue Fluorescence*	200 tests	316	456
21614	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Enhanced Selectivity*	200 tests	370	467
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	1 plate	N/A	560
21612	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	1 plate	N/A	560
21613	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	10 plates	N/A	560
21609	PhosphoWorks™ Luminometric ATP Assay Kit *Steady Glow*	1 plate	N/A	560

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Protein Kinases 8

Protein Kinases

Kinase is a type of enzyme that transfers phosphate groups from high energy donor molecules, such as ATP, to specific substrates, a process referred to as phosphorylation. Kinases are part of the larger family of phosphotransferases. One of the largest groups of kinases is protein kinases, which act on and modify the activity of specific proteins. Kinases are used extensively to transmit signals and control complex processes in cells. More than five hundred different kinases have been identified in humans. Their diversity and their role in signaling make them an object of intensive studies. Quite a few protein kinases have been identified a reliable drug discovery targets. Various other kinases act on small molecules such as lipids, carbohydrates, amino acids, and nucleotides, either for signaling or to prime them for metabolic pathways. The human protein kinase family is divided into the following groups:

- *AGC kinases: containing PKA, PKC and PKG.*
- *CaM kinases: containing the calcium/calmodulin-dependent protein kinases.*
- *CK1: containing the casein kinase 1 group.*
- *CMGC: containing CDK, MAPK, GSK3 and CLK kinases.*
- *STE: containing the homologs of yeast Sterile 7, Sterile 11, and Sterile 20 kinases.*
- *TK: containing the tyrosine kinases.*
- *TKL: containing the tyrosine-kinase like group of kinases.*

All kinases use ATP as their substrate to give ADP as a product. Most of the commercial protein kinase assay kits are based on monitoring either the phosphopeptide formation or the ATP depletion. For the kinase assay kits that are based on the detection of phosphopeptides, one has to spend time and efforts to identify an optimized peptide substrate while the ATP depletion method suffers various interferences due to the use of luciferase that is inhibited or activated by various biological compounds. However, both our ATP and ADP detection kits might be used for monitoring a kinase activity generically.

Amplite™ Universal Fluorimetric Kinase Assay Kit (Cat# 31001) is based on monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity and is measured fluorimetrically. This enzyme-coupled kit provides a fast, simple, and homogeneous assay to measure kinase activities. It is a non-radioactive and no wash method to detect the amount of ADP produced from enzyme reaction. Its characteristics of high sensitivity (<0.3 µM ADP) and broad ATP tolerance (1-300 µM) make it an ideal kit for determining kinase Michaelis-Menten kinetics and for screening and identifying kinase inhibitors. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

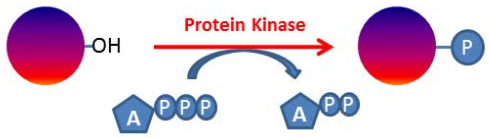

Figure 8.1. Protein kinase reaction scheme.

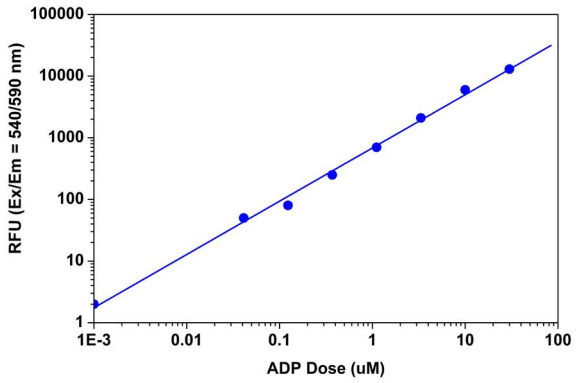

Figure 8.2. ADP dose responses were measured with Amplite™ Universal Fluorimetric Kinase Assay Kit (Cat# 31001) in a 384-well black solid plate. As low as 0.3 µM ADP was detected with 30 minutes incubation.

Table 8.1 Kinase Detection Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
31001	Amplite™ Universal Fluorimetric Kinase Assay Kit *Red Fluorescence*	250 tests	571	585
21655	PhosphoWorks™ Fluorimetric ADP Assay Kit *Red Fluorescence*	100 tests	571	585
21611	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Blue Fluorescence*	200 tests	316	456
21614	PhosphoWorks™ Fluorimetric Pyrophosphate Assay Kit *Enhanced Selectivity*	200 tests	370	467
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	1 plate	N/A	560
21609	PhosphoWorks™ Luminometric ATP Assay Kit *Steady Glow*	1 plate	N/A	560
21612	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	1 plate	N/A	560
21613	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	10 plates	N/A	560

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Transferases 9

Transferase probes and assay kits at-a-glance*

Enzyme	Colorimetric	Fluorimetric
Alanine Amino-transferase	13803	13802
Aspartate Aminotransferase	13801	13800
Histone Deacetylase (HDAC)		13601

* products listed by catalog number

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Transferases

9.1 Alanine Aminotransferase

Alanine aminotransferase (ALT), also called serum glutamate pyruvate transaminase (GPT), is a member of transferase family. It catalyzes the reversible transfer of an α-amino group between alanine and glutamate, and is an important enzyme in amino acid metabolism. ALT is found mainly in liver and small amount in heart, muscle, and kidneys. In healthy subjects, serum ALT levels are low. However, when cells are damaged, such as acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infarction, ALT may leak into the blood stream and the ALT levels are significantly elevated. Therefore, determination of serum ALT level has great clinical and diagnostic significance.

Amplite™ Fluorimetric Alanine Aminotransferase assay kit (Cat# 13802) provides a quick and sensitive method for the measurement of ALT in various biological samples. ALT catalyzes the reaction of alanine and α-ketoglutarate to pyruvate and glutamate:

Alanine (Ala) + α-ketoglutarate ⇌ pyruvate + glutamate (Glu)

The product glutamate is measured by the generation of a red fluorescent product through an enzyme coupled reaction cycle. The signal can be read using a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm). With Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit, as little as 4 mU/mL ALT was detected in a 100 µL reaction volume. The assay is robust, and can be readily adapted for a wide variety of applications.

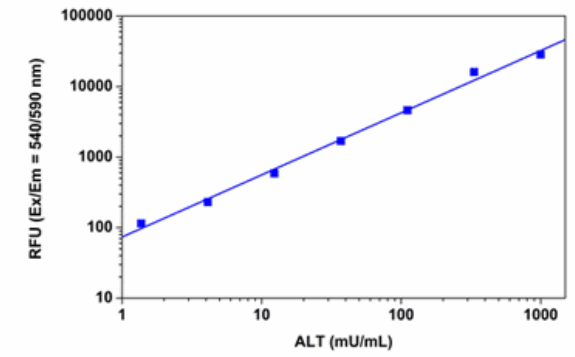

Figure 9.1. ALT dose responses were measured with Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit (Cat# 13802) in a 96 well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 4 mU/mL ALT was detected with 60 minutes incubation (n=3) at 37 ºC.

The product glutamate can also be measured with Amplite™ Colorimetric Alanine Aminotransferase Assay Kit (Cat# 13803) by the generation of a blue color product through an enzyme coupled reaction cycle. The signal can be read using an absorbance microplate reader at ~575 nm or by the absorbance ratio of $A_{570\,nm}$ to $A_{610\,nm}$ to increase assay sensitivity. With Amplite™ Colorimetric Alanine Aminotransferase Assay Kit, as little as 10 mU/mL ALT was detected in a 100 µL reaction volume. The assay is robust, and can be readily adapted for a wide variety of applications.

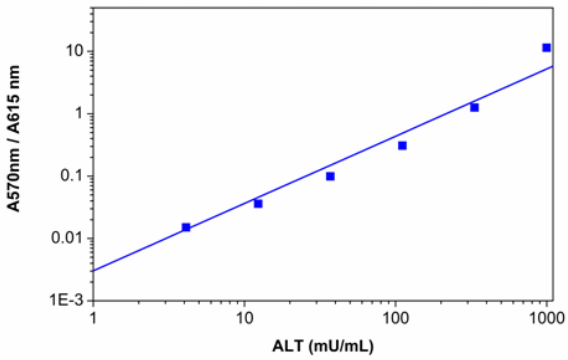

Figure 9.2. ALT dose responses were measured with Amplite™ Colorimetric Alanine Aminotransferase Assay Kit (Cat# 13803) in a 96 well black plate using a SpectrMax microplate reader (Molecular Devices). As low as 10 mU/mL ALT was detected with 60 minutes incubation (n=3) at 37 ºC.

Table 9.1 Alanine Aminotransferase Assay Kits

Cat. #	Product Name	Size
13803	Amplite™ Colorimetric Alanine Aminotransferase Assay Kit *Blue Color*	200 tests
13802	Amplite™ Fluorimetric Alanine Aminotransferase Assay Kit	200 tests

9.2 Aspartate Aminotransferase

Aspartate aminotransferase (AST), also called serum glutamic oxaloacetic transaminase (GOT), is a member of transferase family. It catalyzes the reversible transfer of an α-amino group between aspartate and glutamate, and is an important enzyme in amino acid metabolism. AST is found in many body tissues such as liver, heart, muscle, kidneys and brain. In healthy subjects, serum AST levels are low. However, when cells are damaged, such as acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infarction, AST may leak into the blood stream and the AST levels are significantly elevated. Therefore, determination of serum AST level has great clinical and diagnostic significance.

Amplite™ Fluorimetric Aspartate Aminotransferase (AST) Assay Kit (Cat# 13800) provides a quick and sensitive method for the measurement of AST in various biological samples. Aspartate transaminase catalyzes the reaction of aspartate and α-ketoglutarate to oxaloacetate and L-glutamate:

Aspartate (Asp) + α-ketoglutarate ⇌ oxaloacetate + glutamate (Glu)

The product L-glutamate is measured by the generation of a red fluorescent product through an enzyme coupled reaction cycle. The signal can be read by a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm). With Amplite™ Fluorimetric Aspartate Aminotransferase Assay Kit (Cat# 13800), we were able to detect as little as 2 mU/mL AST in a 100 µL reaction volume. The assay is robust, and can be readily adapted for a wide variety of applications. The assay can be either run in 96-well or 384-well microplate.

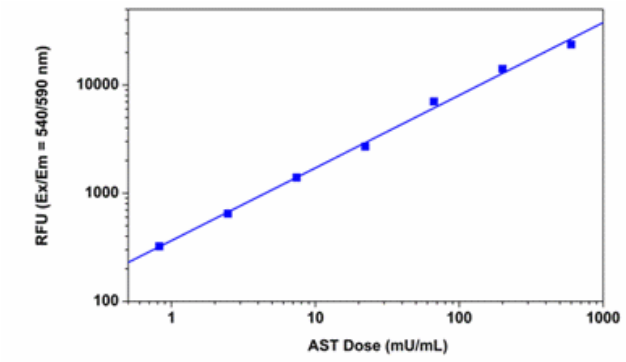

Figure 9.3. AST dose responses were measured with Amplite™ Fluorimetric Aspartate Aminotransferase Assay Kit (Cat# 13800) in a 96 well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 2 mU/mL AST was detected with 60 minutes incubation (n=3) at room temperature.

The product L-glutamate can also be measured with Amplite™ Colorimetric Aspartate Aminotransferase Assay Kit (Cat# 13801) by the generation of a blue color product through an enzyme coupled reaction cycle. The signal can be read using an absorbance microplate reader at ~575 nm or by the absorbance ratio of $A_{570\ nm}$ to $A_{610\ nm}$ to increase assay sensitivity. With Amplite™ Colorimetric Aspartate Aminotransferase Assay Kit, we have detected as little as 3 mU/mL AST in a 100 µL reaction volume. The assay is robust, and can be readily adapted for a wide variety of applications.

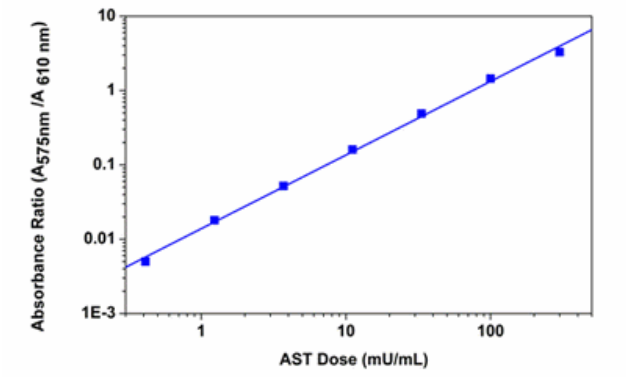

Figure 9.4. AST dose response was measured with Amplite™ Colorimetric Aspartate Aminotransferase Assay Kit (Cat# 13801) in a 96 well clear bottom plate using a SpectraMax microplate reader (Molecular Devices). As low as 3 mU/mL AST was detected with 60 minutes incubation (n=3) at room temperature.

Table 9.2 Aspartate Aminotransferase Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13801	Amplite™ Colorimetric Aspartate Aminotransferase (AST) Assay Kit	200 tests	575	N/A
13800	Amplite™ Fluorimetric Aspartate Aminotransferase (AST) Assay Kit	200 tests	571	585

9.3 HDAC Activity Assay

Histone deacetylases (HDACs) are a class of enzymes that remove acetyl groups from a ε-N-acetyl lysine amino acid on a histone. Deacetylation restores the positive electric charge of the lysine amino acids, which increases the histone's affinity to the negatively charged phosphate backbone of DNA. This process generally down-regulates DNA transcription by blocking the access of transcription factors. HDAC inhibitors are being studied as a treatment for cancers. Amplite™ Fluorimetric HDAC Activity Assay Kit (Cat# 13601) provides a quick, convenient, and sensitive method for the detection of HDAC activity. This kit uses our non-peptide HDAC Green™ substrate that is much more sensitive than the peptide-based HDAC substrates such as Ac-RGK(Ac)-R110, Ac-RGK(Ac)-AMC and Ac-RGK(Ac)-AFC. In addition, HDAC Green™ substrate is also much more resistant to protease hydrolysis than other commercial peptide-based HDAC substrates. Our kit can be used for measuring HDAC activity in cell lysates or HDAC inhibitor screening with cell extracts or purified enzymes. The long wavelength emission of the HDAC Green™ substrate makes the assay less interfered from compounds and cell components. HDAC activity is monitored with excitation at 490 nm and emission at 525 nm.

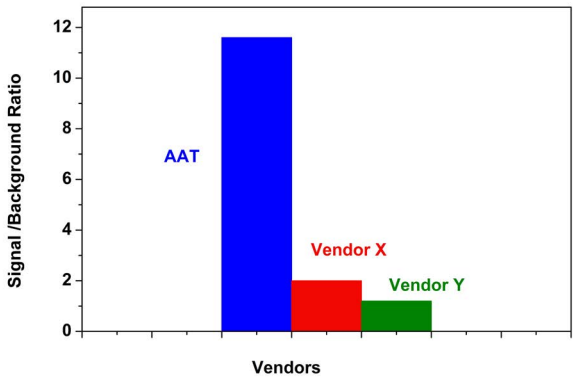

Figure 9.5. HDAC activity in HeLa nuclear extract measured with Amplite™ Fluorimetric HDAC Activity Assay Kit (in blue, Cat# 13601) was compared with Vendor X (in red) and Vendor Y (in green), both of which use Ac-RGK(Ac)-R110 peptide substrate. The signal/background ratio of the HDAC activity measured with Amplite™ Fluorimetric HDAC Activity Assay Kit is more than10 times higher than those of Vendors X and Y.

Table 9.3 HDAC Activity Assay Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13601	Amplite™ Fluorimetric HDAC Activity Assay Kit	200 tests	498	520

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Luciferases 10

luciferase probes and assay kits at-a-glance*

Luciferase	Assay	Emission
Firefly Luciferase	12518	533 nm
Gussia Luciferase	12530	466 nm
Renilla Luciferase	12535	466 nm

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Luciferases

10.1 Firefly Luciferase Reporter Gene Assay

The most versatile and common reporter gene is the luciferase of the North American firefly photinus pyralis. The protein requires no posttranslational modification for enzyme activity. It is not even toxic in high concentration (*in vivo*) and can be used in pro- and eukaryotic cells.

AAT Bioquest Amplite™ Luciferase Reporter Gene Assay Kits use a proprietary DTT-free formulation to quantify luciferase activity in live cells and cell extracts. The assay is based on firefly luciferase, a monomeric 61 kD enzyme that catalyses a two-step oxidation of luciferin, which yields light at 560 nm. Our formulation generates a luminescent product that gives strong luminescence upon interaction with luciferase. The kits provide all the essential components with an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. They have high sensitivity and can be used for the assays that require low detection limit. The kits have a fast, simple, and homogeneous bioluminescence assay for studying gene regulation and function. The assay is compatible with the use of standard cell growth media.

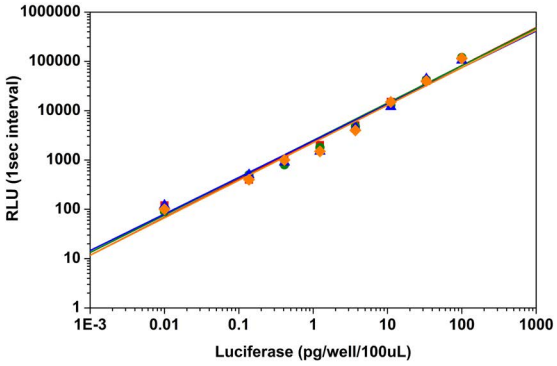

Figure 10.1. Luciferase dose responses were measured with Amplite™ Luciferase Reporter Gene Assay Kit (Cat# 12518). The kit can detect as low as 0.1 pg/well luciferase with 20-minute to 5-hour incubation without losing signal intensity. The integration time is 1 second. The half life is more than 4 hours.

10.2 Gaussia Luciferase Reporter Gene Assay

The most versatile reporter gene is the firefly luciferase. Recently there is steadily increasing use of other luciferases, such as Gaussia luciferase, since these reporters are smaller and do not require the presence of ATP. Gaussia luciferase is a 20 kD protein which catalyzes coelenterazine oxidation by oxygen to produce light. The bioluminescent enzyme derived from the marine copepod Gaussia princeps is efficiently secreted from mammalian cells upon expression.

AAT Bioquest Amplite™ Gaussia Luciferase Reporter Gene Assay Kits use a proprietary luminogenic formulation to quantify luciferase activity in cell medium. The formulation generates a luminescent product that gives strong luminescence upon interaction with Gaussia luciferase. The kits provide all the essential components that are compatible with HTS liquid handling instruments. They have high sensitivity and can be performed in convenient 96-well and 384-well microtiter-plate formats. The "glow-type" signal with a half-life of one hour provides a consistent signal across large number of assay plates. The assay is compatible with standard cell growth media.

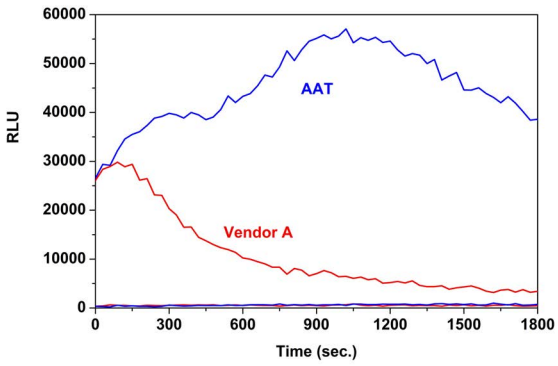

Figure 10.2. Secreted Gaussia luciferase culture medium was measured with Amplite™ Gaussia Luciferase Reporter Gene Assay Kit (blue line, Cat# 12530) and a commercially available Gaussia Luciferase Assay Kit (red line) respectively in a 96-well white plate using a NOVOstar plate reader (BMG Labtech).

Table 10.1 Firefly and Gaussia Luciferase Reporter Gene Assay Probes and Kits

Cat. #	Product Name	Size	Em (nm)
12530	Amplite™ Gaussia Luciferase Reporter Gene Assay Kit	1 plate	466
12518	Amplite™ Luciferase Reporter Gene Assay Kit *Bright Glow*	1 plate	466
12535	Amplite™ Renilla Luciferase Reporter Gene Assay Kit	1 plate	466
12501	D-Luciferin, Free Acid *UltraPure Grade*	25 mg	533
12505	D-Luciferin, Potassium Salt *UltraPure Grade*	25 mg	533
12509	D-Luciferin, Sodium Salt *UltraPure Grade*	25 mg	533
12500	Luciferase *Recombinant Firefly*	1 mL	N/A

10.3 Renilla Luciferase Reporter Gene Assay

Common reporter genes include beta-galactosidase, beta-glucuronidase and luciferase. The most versatile reporter gene is the firefly luciferase. Recently there is steadily increasing use of other luciferases, such as Renilla luciferase, since these reporters are smaller and do not require the presence of ATP.

Amplite™ Renilla Luciferase Reporter Gene Assay Kits are designed to provide a fast and sensitive method to detect the luciferase from sea pansy (Renilla reniformis). They use a proprietary luminogenic formulation to quantify Renilla luciferase activity in cell-based assays. Our formulation generates a luminescent product that gives strong luminescence upon interaction with Renilla luciferase. The kits provide all the essential components. They have high sensitivity and can be performed in a convenient 96-well and 384-well microtiter-plate format. The "glow-type" signal with a half-life of one hour provides a consistent signal across large number of assay plates. The assay is compatible with standard cell growth media. The kits enable the measurement of primary expression or gene expression with wild type and synthetic *hRluc* genes .

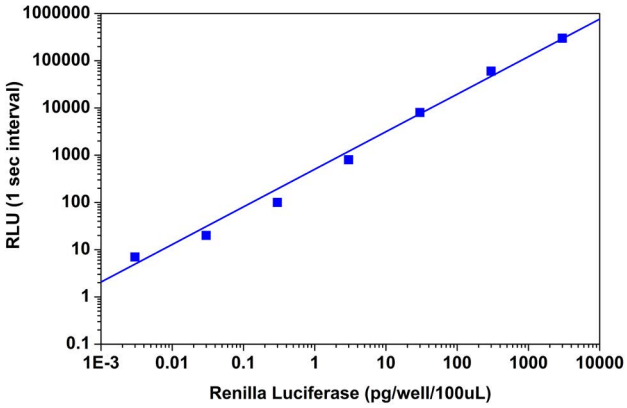

Figure 10.3. Renilla luciferase dose responses were measured with Amplite™ Renilla Luciferase Reporter Gene Assay Kit (Cat# 12535) in a 96-well solid black plate with a NOVOstar plate reader (BMG Labtech). As low as 1pg/mL (0.1pg/well/100uL) Renilla luciferase was detected with 30-minutes incubation (n=3).

Table 10.2 Renilla Luciferase Reporter Gene Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
12535	Amplite™ Renilla Luciferase Reporter Gene Assay Kit *Bright Glow*	1 plate	429	466
12536	Amplite™ Renilla Luciferase Reporter Gene Assay Kit *Bright Glow*	10 plates	429	466
12537	Amplite™ Renilla Luciferase Reporter Gene Assay Kit *Bright Glow*	100 plates	429	466

Table 10.3 Specific Luciferase Specificity, Cofactor Requirements and Physical Characteristics

Organism	Luciferase	Size (kDa)	Substrate	Requires	Secreted
Photinus pyralis	North American firefly luciferase	61	D-luciferin	Mg, ATP	No
Luciola cruciata	Japanese firefly (Genji-botaru) luciferase	64	D-luciferin	Mg, ATP	No
Luciola italica	Italian firefly Luciferase	64	D-luciferin	Mg, ATP	No
Luciola lateralis	Japanese firefly (Heike) luciferase	64	D-luciferin	Mg, ATP	No
Luciola mingrelica	East European firefly luciferase	64	D-luciferin	Mg, ATP	No
Photuris pennsylvanica	Pennsylvania firefly luciferase	64	D-luciferin	Mg, ATP	No
Pyrophorus plagiophthalamus	Click beetle luciferase	64	D-luciferin	Mg, ATP	No
Phrixothrix hirtus	Railroad worm luciferase	64	D-luciferin	Mg, ATP	No
Renilla reniformis	Renilla luciferase	36	Coelenterazine	N/A	No
	Rluc8 (mutant of Renillaluciferase)	36	Coelenterazine	N/A	No
	Green Renilla luciferase	36	Coelenterazine	N/A	No
Gaussia princeps	Gaussia luciferase	20	Coelenterazine	N/A	Yes
	Gaussia-Dura luciferase	20	Coelenterazine	N/A	Yes
Cypridina noctiluca	Cypridina luciferase	62	Vargulin/Cypridinaluciferin	N/A	Yes
Cypridina hilgendorfii	Cypridina (Vargula) luciferase	62	Vargulin/Cypridinaluciferin	N/A	Yes
Metridia longa	Metridia luciferase	23.8	Coelenterazine	N/A	Yes
Oplophorus gracilorostris	OLuc	19	Coelenterazine	N/A	Yes

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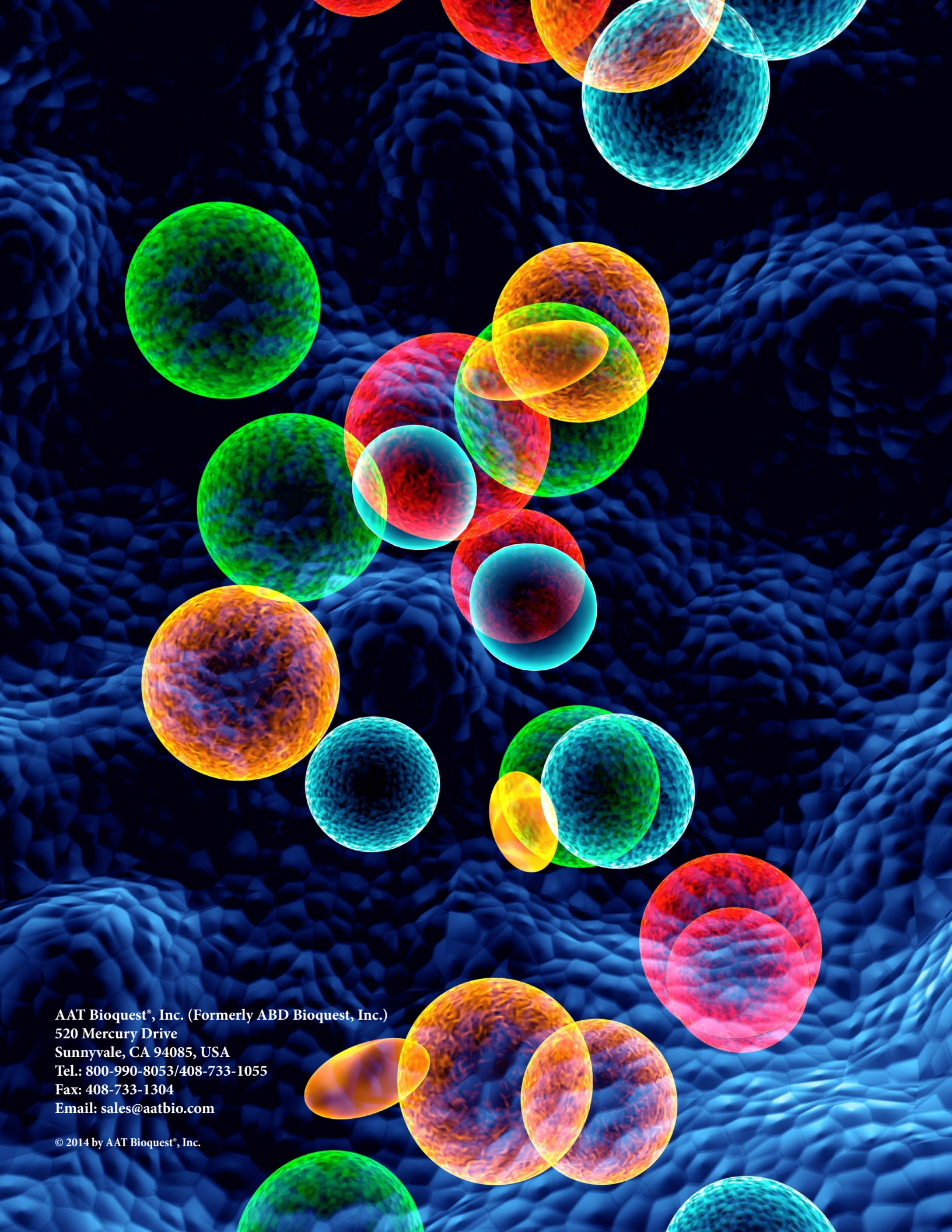

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