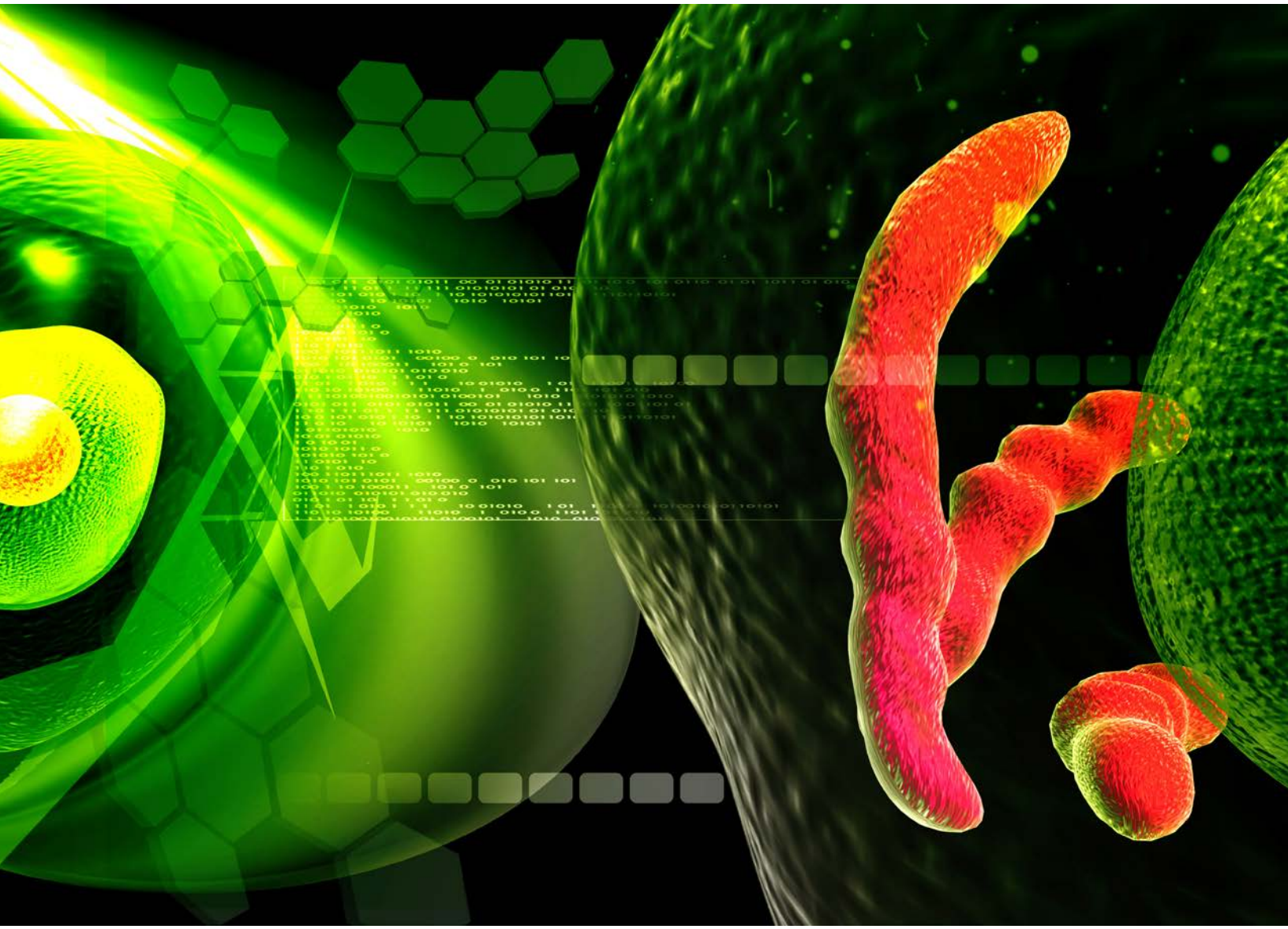
Labeling Antibodies and Biopolymers



Fluorescent Labeling Dyes | **Protein Labeling Kits** | **Buccutite™ Crosslinking Technology**

ImTec DIAGNOSTICS NV

AAT Bioquest®

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. 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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CUSTOMER SERVICE & ORDERING INFORMATION

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BD Horizon™ (BD Biosciences)
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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.

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Optimized Fluorescent Labeling Solutions

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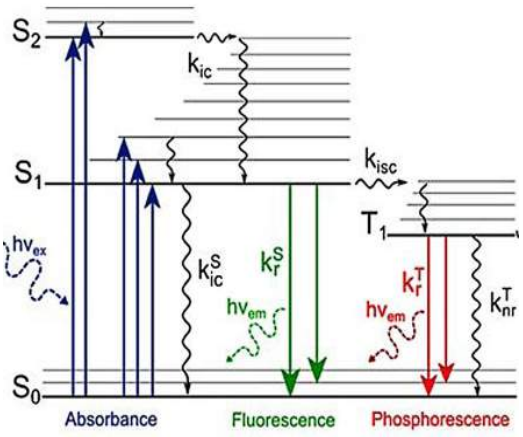

Figure 1.1 The Jablonski diagram illustrating the process of fluorescence.

Fluorescence is light emitted by a substance in response to shorter wavelength radiation, such as by UV-light. It typically involves a three stages (see Figure 1.1).

1. **Stage 1:** Excitation – a photon of energy, being supplied by either a lamp or a laser, is absorbed by the fluorophore. If the energy absorbed is sufficient, electrons in the molecule transition from a lower energy level (ground state) to a higher energy level (excited state).
2. **Stage 2:** Excited-State Lifetime – once in the excited-state, there are multiple energy levels the fluorophore can obtain depending on the initial energy provided by the external light source. Fluorophores are relatively unstable at high energy configurations and must dissipate energy until it adopts the lowest energy level in the excited state, where it is semi-stable.
3. **Stage 3:** Fluorescence Emission – the fluorophore undergoes conformation changes until it transitions back to ground state. During this stage, excess energy is released and emitted as light. The emitted light is typically of lower energy thus resulting in a longer wavelength.

By taking advantage of the fluorescent properties of certain compounds, called fluorophores, probes can be designed to study biological samples. For example, by attaching fluorophores to a target molecule researchers are able to detect complex biomolecular assemblies. Reactive fluorescent dyes are widely used to modify peptides, proteins (in particular, antibodies), oligonucleotides, nucleic acids, carbohydrates and other biological molecules. In general, the preferred bioconjugates should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules.

A number of fluorescent dyes have been developed and commercialized for labeling biomolecules. One of the most popular is fluorescein isothiocyanate (FITC). FITC, however, has certain limitations such as pH dependence, low photostability and short wavelengths associated with its use. An alternative to these classic dyes, such as fluorescein and rhodamine, is the Alexa Fluor® dyes, which have been used for labeling proteins and other biomolecules because of their improved spectral properties. However, the extraordinarily high cost of Alexa Fluor® dyes prevents their use in applications which require a large amount of dye such as labeling peptides and oligonucleotides. In addition, Alexa Fluor® dyes do not provide a significant benefit for labeling peptides, oligo and other small molecules.

AAT Bioquest offers an affordable, comprehensive line of iFluor™, mFluor™ and trFluor™ labeling dyes tiered and optimized for a variety of applications. iFluor™ dyes demonstrate strong fluorescence, high photostability and pH independence when conjugated to proteins and other biopolymers. This is because the iFluor™ dyes are optimized for labeling proteins, antibodies and other biopolymers such as nucleic acids. Our mFluor™ fluorescent labeling dyes are specifically developed for flow cytometry-based applications. Our trFluor™ dyes are ideal for TR-FRET-based applications and other time-resolved fluorescence-based assays with superior performance and reduced cost.

To label peptides, oligonucleotides and other small molecules, Tide Fluor™ dyes are the best choice. On peptides, oligonucleotides and other small molecules, these

Optimized Fluorescence Labeling Solutions Offered by AAT Bioquest

iFluor™ Dyes:

- **Research Target:** Antibodies, Proteins, Nucleic Acids
- **Benefit:** Superior performance compared to classic dyes with a fraction of the cost (compared to Alexa Fluor® and DyLight™ dyes)

Tide Fluor™ Dyes:

- **Research Target:** Oligonucleotides and Peptides
- **Benefit:** Better performance compared to classic dyes with a fraction of the cost (compared to Alexa Fluor® and DyLight™ dyes)

mFluor™ Dyes:

- **Research Target:** Flow cytometry applications
- **Benefit:** Enable multicolor detection, optimized for flow cytometry

trFluor™ Dyes:

- **Research Target:** TR-FRET applications
- **Benefit:** No enhancers required, multiple reactive forms available

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Tide Fluor™ dyes perform as well as Alexa Fluor® dyes but with significant savings. To develop FRET and TR-FRET assays, our Tide Quencher™ non- fluorescent labeling dyes are excellent quenchers. The Tide Quencher™ dyes span the full visible spectrum and thus can be selected to pair with essentially all the existing fluorescent donor dyes including DyLight™, Alexa Fluor®, and cyanine dyes.

Amine-Reactive Fluorescent Dyes

Amine-reactive fluorescent dyes are widely used to modify proteins, nucleic acids, and other biomolecules. This is due to the abundant quantities of amine groups naturally present in biomolecules, and the ability to easily introduced amine groups into a biomolecule. Conjugates prepared with amine reactive dyes are routinely used for immunochemistry, fluorescence in situ hybridization (FISH), cell tracing, and other biological applications. Of the major classes of amine-reactive fluorescent reagents currently used to label biopolymers, succinimidyl esters (SE) are predominantly used. AAT Bioquest offers all the popular amine-reactive fluorescent dyes for peptide or protein labeling, nucleotide modifications and microarray applications.

Carboxyl-Reactive Labeling Dyes

Succinimidyl esters (SE) are proven to be the best reagents for amine modifications because the amide bonds formed are essentially identical to, and as stable as, natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines.

A few factors should be considered when SE compounds are used for conjugation reactions:

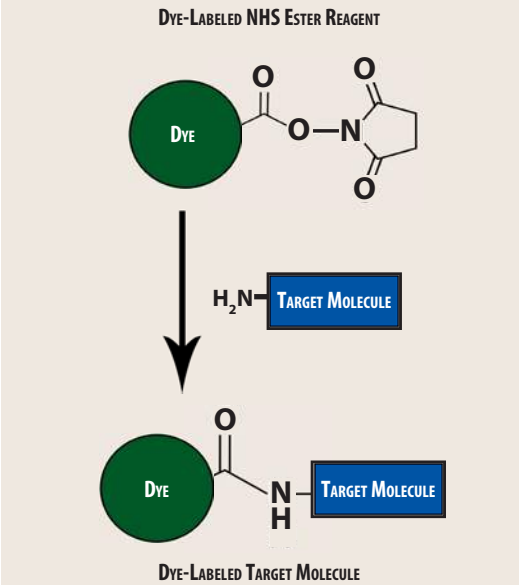
1. **Reaction Solvents:** For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethyl sulfoxide (DMSO).
2. **Reaction pH**: The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Thus amine acylation reactions are usually carried out above pH 7.5. Protein modifications by succinimidyl esters can typically be done at pH 8.5 - 9.3.
3. **Reaction Buffers:** Buffers that contain free amines such as Tris, glycine and thiol compounds must be avoided when using amine-reactive reagents. Ammonium salts, such as ammonium sulfate and ammonium acetate that are widely used for protein precipitation must also be dialyzed before performing dye conjugations.
4. **Reaction Temperature:** Most of the conjugations are performed at room temperature. However, elevated or reduced temperatures may be required for a particular labeling reaction.

For labeling biopolymers, it is quite critical to properly control the degree of substitution (DOS). A high degree of labeling may significantly decrease the water solubility and binding affinity or specificity of the final biomolecule conjugates. Although fluorescent labeling of biomolecules is relatively straightforward, preparing

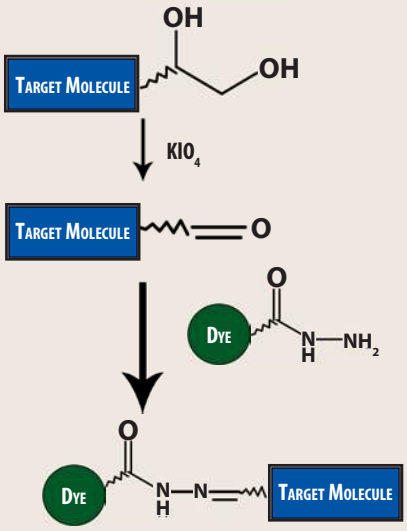
Table 1.1 Popular labeling chemistries of fluorescent reative dyes.

Reactivity Class	Target Functional Group	Reactive Chemical Group
Amine-reactive	- NH_2	NHS ester Imidoester Pentafluorophenyl ester Hydroxymethyl phosphine
Carboxyl-to-amine reactive	-COOH	Carbodiimide (e.g., EDC)
Sulfhydryl-reactive	-SH	Maleimide Haloacetyl (Bromo- or Iodo-) Pyridyldisulfide Thiosulfonate Vinylsulfone
Aldehyde-reactive i.e., oxidized sugars (carbonyls)	-CHO	Hydrazide Alkoxyamine
Hydroxyl (nonaqueous)-reactive	-OH	Isocyanate
Azide-reactive	$-N_3$	Phosphine

Amine-Reactive Labeling Chemistry (NHS Ester)



Carbonyl-Reactive (Amine-Containing) Labeling Chemistry



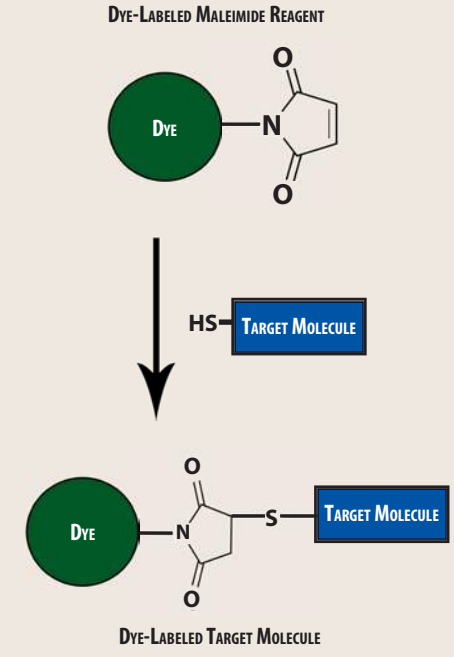
the optimal conjugate may require extensive experimentation.

Amine-containing dyes can also be used in conjunction with water-soluble carbodiimides, such as EDC, to react with carboxyl groups found on biopolymers. Such reactions result in an amide bond formation between the biopolymer and the amine-reactive dye. Either NHS or Sulfo-NHS may be used to improve the coupling efficiency of EDC-mediated protein–carboxylic acid conjugations. A large excess of the amine-containing dyes are usually used for EDC-mediated bioconjugations in concentrated protein solutions at low pH to reduce intra- and inter-protein coupling to lysine residues, a common side reaction.

Carbonyl-Reactive Dyes and Their Applications

Amine-containing dyes can be used to modify water-soluble biopolymers such as proteins through the formation of Schiff Base or reductive amination. Periodate-oxidation introduces aldehydes and ketones into the biopolymers for subsequent reductive amination which can be used to modify carbohydrates, glycoproteins and nucleic acids. The combination of periodate oxidation and reductive amination provides an effective way for site-selective modifications of biopolymers. For example, periodate oxidation of 3'-terminal ribose is reported to be one of the few methods of selectively modifying RNA. Periodate-oxidized ribonucleotides are converted to fluorescent nucleotide probes by reacting with fluorescent hydrazines and amines.

Thiol Reactive Labeling Chemistry (Maleimide)



Thiol-Reactive Fluorescent Dyes

Free thiol groups are less abundant in molecules compared to amine groups but are often exploited as a means of site specific modification of a molecule. Therefore thiol-reactive dyes are often used to prepare fluorescent peptides, proteins and oligonucleotides for probing biological structures, functions and interactions. For example, thiol-reactive dyes have been used to develop probes for analyzing the topography of proteins in biological membranes, determining distances of specific sequences within the protein or between the proteins, and monitoring the changes in protein conformation using environment-sensitive probes. There are many types of thiol-reactive dyes reported in the literature, including iodoacetamides, disulfides, maleimides, vinyl sulfones as well as various electron-deficient aryl halides and sulfonates.

Maleimides

Maleimides and iodoacetamides are by far the most popular thiol-reactive moieties. The conjugation conditions required by maleimides are less stringent than those of iodoacetamides. Even under neutral conditions, maleimides readily react with thiol moieties of biopolymers to form thioether conjugates (the thioether bond formed is quite stable). Maleimides are generally much less light-sensitive than iodoacetamides. The iodoacetamide compounds are known to be very light liable, especially in solution. Unlike iodoacetamides, maleimides do not react with histidine and methionine under physiological conditions. Most conjugations can be done at room temperature and at a neutral pH. However, elevated or reduced pH or temperatures may be required for certain labeling reaction.

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iFluor™
Fluorescent Labeling Dyes

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Common Excitation Sources Used in Fluoresence Instruments

Light Source	Principal Excitation Lines (nm)
• Mercury Arc Lamp	366, 405, 436, 546, 578
• Xenon Arc Lamp	250-1000
• Tungsten–Halogen Lamp	350-1000
• Blue Diode Laser	405
• Helium–Cadmium Laser	325, 442
• Argon Ion Laser	457, 488, 514
• Nd:YAG Laser	532
• Helium–Neon Laser	543, 594, 633
• Yellow Diode Laser	561
• Krypton Ion Laser	568, 647
• Red Diode Laser	635

Table 2.1 Spectral comparison of all iFluor™ labeling dyes.

Fluorophore	Replaces	Excitation/Emission (nm)
iFluor™ 350	Alexa Fluor® 350, DyLight™ 350, AMCA	345/442
iFluor™ 405	Alexa Fluor® 405, DyLight™ 405	401/420
iFluor™ 488	Alexa Fluor® 488, DyLight™ 488	491/514
iFluor™ 514	Alexa Fluor® 514	518/542
iFluor™ 532	Alexa Fluor® 532	531/556
iFluor™ 546	Alexa Fluor® 546	541/557
iFluor™ 555	Alexa Fluor® 555, DyLight™ 550, Cy3®, TRITC	555/565
iFluor™ 568	Alexa Fluor® 568	568/587
iFluor™ 594	Alexa Fluor® 594, DyLight™ 594, Texas Red®	594/614
iFluor™ 610	Alexa Fluor® 610	605/627
iFluor™ 633	Alexa Fluor® 633, DyLight™ 633	638/655
iFluor™ 647	Alexa Fluor® 647, DyLight™ 650, Cy5®	649/665
iFluor™ 680	Alexa Fluor® 680, DyLight™ 680, Cy5.5®, IRDye® 700	676/695
iFluor™ 700	Alexa Fluor® 700	685/710
iFluor™ 750	Alexa Fluor® 750, DyLight™ 750, Cy7®	749/775
iFluor™ 790	Alexa Fluor® 790, DyLight™ 800, IRDye® 800	782/811
iFluor™ 800	No other commercial equivalents	801/820
iFluor™ 810	No other commercial equivalents	811/825
iFluor™ 820	No other commercial equivalents	824/831
iFluor™ 860	No other commercial equivalents	863/868

iFluor™ dyes are a series of water-soluble, fluorescent labeling dyes that span the full UV-visible-IR spectrum. Their hydrophilic nature allows protein conjugation to be readily performed in an aqueous media. This minimizes the use of organic solvents, resulting in conjugates that are resistant to precipitation during storage. iFluor™ dyes have improved labeling performance compared to the classic fluorescent labeling dyes such as FITC, TRITC, Texas Red® and cyanine dyes, and significantly outperform their corresponding Alexa Fluor® labeling dyes in certain labeling applications.

Other features of our iFluor™ dyes include:

- iFluor™ dyes are available in a variety of reactive forms such as amine reactive or thiol reactive.
- iFluor™ conjugates exhibit more intense fluorescence than other spectrally similar conjugates of classic fluorescent dyes such as FITC, TAMRA and ROX under similar excitations.
- iFluor™ dyes are more photostable than the classic fluorescent dyes such as FITC and Cy dyes.
- iFluor™ dyes have absorption spectra that match the principal output wavelengths of common excitation sources (such as 488 nm, 555 nm, 633 nm and 647 nm) as shown in Table 2.1.
- iFluor™ dyes and their conjugates are available in a variety of distinct fluorescent colors.
- iFluor™ dyes are robust and highly fluorescent over a broad pH range with little pH sensitivity.

Our iFluor™ dyes are the result of focused investment into research and development. We are proud of our rapidly expanding product lines, designed to meet constantly changing research needs. Our focus on innovation has allowed us to solve many of the limitations faced by existing fluorescent labeling technologies. This fact, combined with our extensive library of classic fluorescent labeling reagents, allows us to provide the most comprehensive set of labeling tools available on the market. With high quality products, and competitive pricing, AAT Bioquest offers an unmatched catalog of products and a valued customer experience.

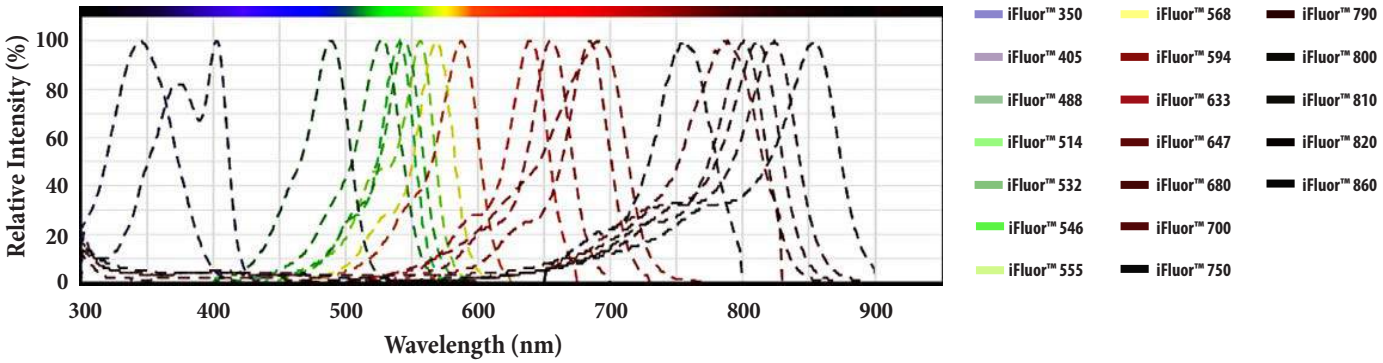

Figure 2.1 Excitation Spectrum of iFluor™ Labeling Dyes

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Blue Fluorescent Dyes

iFluor™ 350

A Superior Replacement for Alexa Fluor® 350, DyLight™ 350 and AMCA Dyes

Although AMCA is the predominant labeling dye for preparing blue fluorescent protein conjugates, AMCA has poor water solubility. iFluor™ 350 dyes are an affordable superior replacement for AMCA and Alexa Fluor® 350. iFluor™ 350 dyes have spectral properties essentially identical to those of AMCA, DyLight™ 350 and Alexa Fluor® 350 dyes. Protein conjugates prepared with iFluor™ 350 dyes are bright, and their fluorescence is not affected by pH in the physiological range (pH 4-10). This pH insensitivity makes iFluor™ 350 dyes useful for assays requiring extreme pH. Under identical test conditions, iFluor™ 350 secondary antibody conjugates gave equivalent or higher signal-to-background ratios than the corresponding Alexa Fluor® 350-labeled conjugates. As one of the brightest fluorescent dye conjugates available in the blue channel, these iFluor™ 350 conjugates are best used for high-abundance targets like actin or tubulin. They are commonly used with iFluor™ 488, 594, and 647 dyes for multiplexing applications.

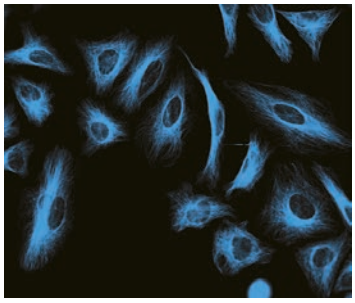

Figure 2.2 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 350 goat anti-mouse IgG (H&L) (Blue, Cat# 16520).

iFluor™ 405

A Superior Replacement for Alexa Fluor® 405 and Cascade Blue® Dyes

iFluor™ 405 dyes are an excellent replacement for Alexa Fluor® 405 and Cascade Blue® dyes because of their nearly identical spectral properties. Protein conjugates prepared with iFluor™ 405 dyes are bright, and their fluorescence is not significantly affected by pH in the physiological range (pH 4-10). iFluor™ 405 dyes and their conjugates are excellent violet laser reagents for flow cytometry research. Besides iFluor™ 405 violet laser flow cytometry dyes, we offer a line of multi-color mFluor™ violet laser flow cytometry reagents labeling dyes and kits (see Pages 18-20).

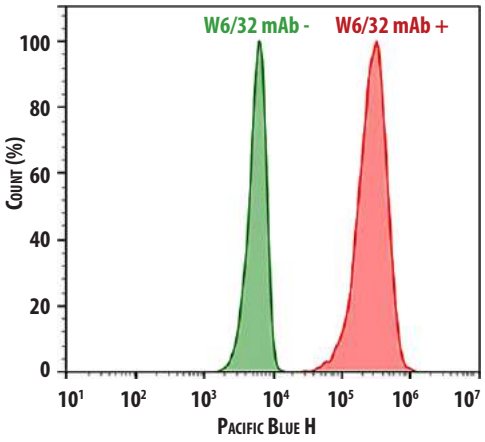

Figure 2.3 HL-60 cells were incubated with (Red, +) or without (Green, -) Anti-human HLA-ABC (W6/32 mAb), followed by iFluor™ 405 goat anti-mouse IgG (H&L) (Cat# 16444). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the Pacific Blue channel (Ex/Em=405/445 nm).

iFluor™ 405-labeled biological conjugates have blue fluorescence with an excitation optimized for the 405 nm violet laser line common in new flow cytometers. iFluor™ 405 conjugates have an excitation and emission spectra nearly identical to Alexa Fluor® 405 and BD's Horizon™ BV421 conjugates. These spectral similarities make iFluor™ 405 dyes an excellent alternative to the corresponding Alexa Fluor® 405 conjugates. Compared to other iFluor™ dye conjugates, iFluor™ 405 dye conjugates have moderate photostability and are commonly used with iFluor™ 488, 594, and

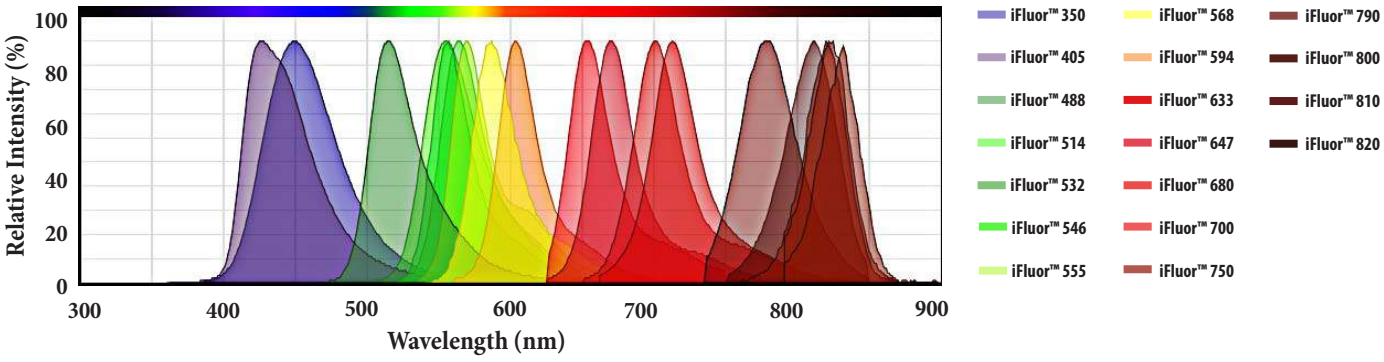

Figure 2.4 Emission Spectrum of iFluor™ Labeling Dyes

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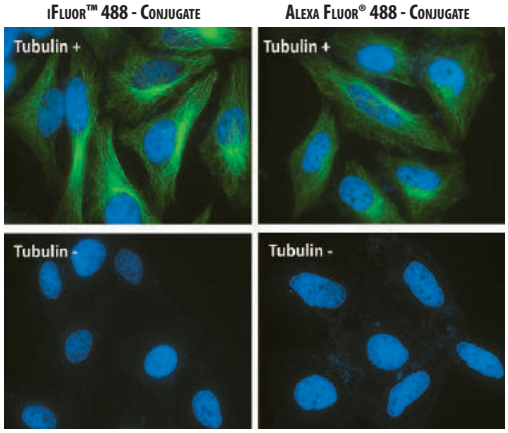

Figure 2.5 Images of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 488 goat anti-mouse IgG (H&L) (Green, Left, Cat# 16528) or Alexa Fluor® 488 goat anti-mouse IgG (Green, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

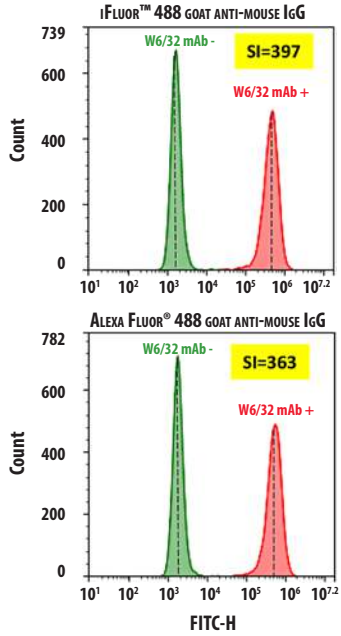

Figure 2.6 HL-60 cells were incubated with (Red, +) or without (Green, -) Anti-human HLA-ABC (W6/32 mAb), followed by iFluor™ 488 goat anti-mouse IgG (Cat# 16528) or Alexa Fluor® 488 goat anti-mouse IgG, respectively. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in FITC channel. The stain index (SI) of each conjugate was calculated. Results indicate that iFluor™ 488 is an excellent replacement for Alexa Fluor® with a slighty better signal-to-noise ratio.

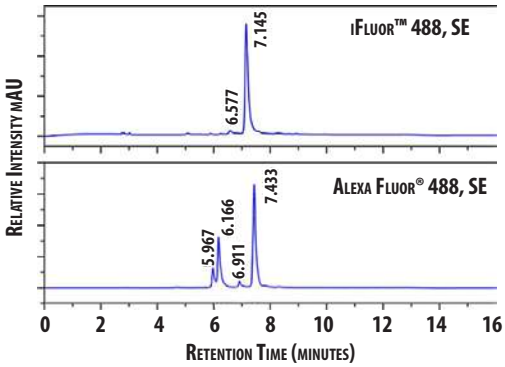

Figure 2.7 HPLC chromatogram comparison of iFluor™ 488 SE (top graph, Cat# 1023) and Alexa Fluor® 488 SE (bottom graph) indicated iFluor™ 488 SE had much higher purity and lot-to-lot reproducibility.

647 dyes for multiplexing applications. As one of the brightest fluorescent dye conjugates available in the blue channel, iFluor™ 405 conjugates are best suited for high-abundance targets like actin or tubulin. In many cases, our iFluor™ 405 labeled conjugates are much brighter than the spectrally similar dye conjugates such as Alexa Fluor® 405 conjugates.

Green Fluorescent Dyes

iFluor™ 430

A Superior Replacement for Alexa Fluor® 430 Dyes

iFluor™ 430 is a bright, green-fluorescent dye that has nearly identical excitation and emission spectra to those of Alexa Fluor® 430. It has a large Stokes Shift (>100 nm) with pH-insensitive fluorescence from pH 4-10. The iFluor™ 430 dyes are an excellent alternative for Alexa Fluor® 430 dyes. NHS or succinimidyl esters are the most popular tool for conjugating this dye to a protein or an antibody. The NHS esters can be used to label the primary amines ($R\text{-}NH_2$) of proteins, amine-modified oligonucleotides, and other amine-containing molecules. Antibody conjugates of iFluor™ 430 can be used for their stable signal generation in imaging and flow cytometry. iFluor™ 430 conjugates exhibit brighter fluorescence and greater photostability than the conjugates of other spectrally similar fluorophores.

iFluor™ 488

A Superior Replacement for Alexa Fluor® 488, DyLight 488™ and FITC Dyes

Although FITC is still the most popular fluorescent labeling dye for preparing green fluorescent bioconjugates, there are certain limitations with FITC, such as severe photobleaching for microscope imaging and pH-sensitive fluorescence. Protein conjugates prepared with iFluor™ 488 dyes are far superior compared to the corresponding FITC conjugates. iFluor™ 488 conjugates are significantly brighter and much more photostable than FITC conjugates. Additionally, the fluorescence of iFluor™ 488 is not affected by pH (4-10). This pH insensitivity is a major improvement over FITC, which emits its maximum fluorescence only at pH above 9.

iFluor™ 488 has spectral properties essentially identical to Alexa Fluor® 488. iFluor™ 488 antibody conjugates have an excitation ideally suited for the 488 nm laser line, making them alternatives to the corresponding Alexa Fluor® 488 and FITC-labeled conjugates. Under the same conditions, iFluor™ 488 labeled antibodies gave equivalent or higher signal-to-background ratios than the corresponding Alexa Fluor® 488-labeled antibodies. Compared to the Alexa Fluor® 488 conjugates which are prepared with the mixed isomers of rhodamine 110, iFluor™ 488 conjugates are prepared using a highly purified single rhodamine isomer. This makes the iFluor™ 488 conjugates much more consistent from lot to lot.

iFluor™ 514

An Excellent Replacement for Alexa Fluor® 514 and Rhodamine 6G Dyes

iFluor™ 514 is a bright, green-fluorescent dye. It is water soluble and pH-insensitive from pH 4-10, making it a superior replacement for rhodamine 6G. The bioconjugates

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prepared with iFluor™ 514 dye can be used in imaging and flow cytometry. The NHS or succinimidyl ester of iFluor™ 514 is the most popular tool for conjugating this dye to a protein or an antibody. NHS esters can be used to label the primary amines ($R\text{-}NH_2$) of proteins, amine-modified oligonucleotides, and other amine-containing molecules. The resulting iFluor™ 514 conjugates exhibit brighter fluorescence and greater photostability than conjugates of other spectrally similar fluorophores such as rhodamine 6G.

iFluor™ 514 has spectral properties almost identical to Alexa Fluor® 514. iFluor™ 514 antibody conjugates have an excitation close to the 488 nm laser line, making them alternatives to the corresponding Alexa Fluor® 514 antibody conjugates.

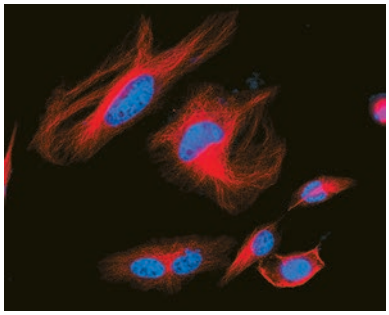

Figure 2.8 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 514 goat anti-mouse IgG (H&L) (Red, Cat# 16532) and nuclei were stained with DAPI (Blue, Cat# 17510).

Yellow Fluorescent Dyes

iFluor™ 532

An Excellent Replacement for Alexa Fluor® 532 Dyes

iFluor™ 532 is a bright, yellow-fluorescent dye. All the iFluor™ 532 dyes are water soluble and their fluorescence is pH-insensitive from pH 4-10. The bioconjugates prepared with iFluor™ 532 dyes are used in imaging and flow cytometry. iFluor™ 532 dyes are optimized to be maximally excited by either the frequency-doubled Nd-Yag laser at 532 nm or the newer InGaN green diode green laser at 536 nm, both of which are increasingly being used in sequencing instruments. The NHS or succinimidyl ester of iFluor™ 532 is the most popular tool for conjugating this dye to a protein or an antibody. NHS esters can be used to label the primary amines ($R\text{-}NH_2$) of proteins, amine-modified oligonucleotides, and other amine-containing molecules. iFluor™ 532 labeled conjugates have spectral properties almost identical to Alexa Fluor® 532, making them alternatives to the corresponding Alexa Fluor® 514-labeled conjugates.

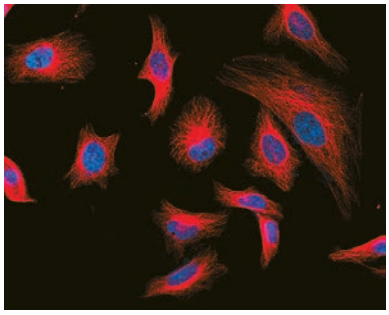

Figure 2.9 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 532 goat anti-mouse IgG (H&L) (Red, Cat# 16536) and nuclei were stained with DAPI (Blue, Cat# 17510).

Orange Fluorescent Dyes

iFluor™ 546

A Superior Replacement for Alexa Fluor® 546 Dyes

iFluor™ 546 is a bright, orange-fluorescent dye with spectral characteristics similar to those of Alexa Fluor® 546. iFluor™ 546 dyes have an excitation at 546 nm and emission at 614 nm when conjugated to proteins. iFluor™ 546 dyes are water soluble and their fluorescence is pH-insensitive from pH 4-10. iFluor™ 546 dyes are optimized for excitation with the 546 nm fiber laser, and can also be well excited by the 532 nm diode lasers that are frequently used in fluorescence sequencing instruments such as Nd:YAG laser (~532 nm), and Helium-Neon laser (~543 nm). Compared to Alexa Fluor® 546, iFluor™ 546 dyes are brighter and better excited at 546 nm, producing a superior signal-to-background ratio (see Fig. 2.10). Additionally, our iFluor™ 546 streptavidin conjugates provide a high fluorescence intensity and low background as validated in immunofluorescence staining of mammalian cells.

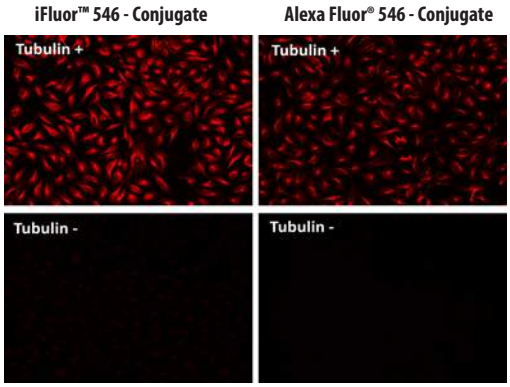

Figure 2.10 Images of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 546 goat anti-mouse IgG (H&L) (Left, Cat# 16536) or Alexa Fluor® 546 goat anti-mouse IgG (Right), respectively.

iFluor™ 555

A Superior Replacement for Alexa Fluor® 555, DyLight™ 550 and Cy3® Dyes

iFluor™ 555 is a bright orange fluorescent labeling dye. Cy3® and Alexa Fluor® 555 are

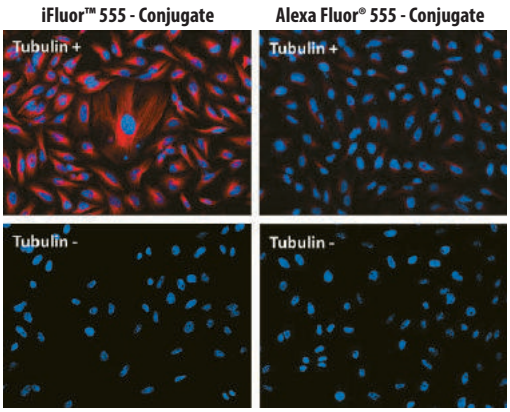

Figure 2.11 Images of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 555 goat anti-mouse IgG (H&L) (Red, Left, Cat# 16540) or Alexa Fluor® goat anti-mouse IgG (Red, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

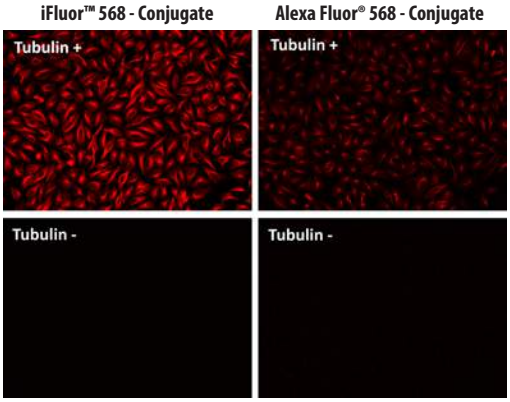

Figure 2.12 Images of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin followed by iFluor™ 568 goat anti-mouse IgG (H&L) (Left, Cat# 16536) or Alexa Fluor® 568 goat anti-mouse IgG (Right), respectively.

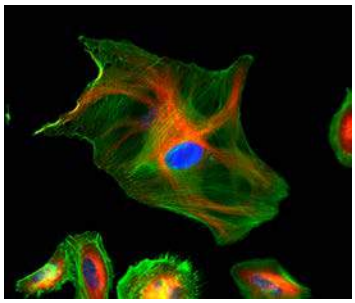

Figure 2.13 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 594 goat anti-mouse IgG (H&L) (Red, Cat# 16548); actin filaments were stained with Phalloidin-iFluor™ 488 Conjugate (Green, Cat# 23115); and nuclei were stained with DAPI (Blue, Cat# 17507).

considered to be the preferred dyes for preparing orange fluorescent bioconjugates. However, when compared to the Cy3® and Alexa Fluor® 555 antibody conjugates, the iFluor™ 555 antibody conjugates are brighter with a higher signal-to-background ratio (see Figure 2.11). The spectral properties of iFluor™ 555 conjugates are nearly identical to those of Cy3® and Alexa Fluor® 555 conjugates, making iFluor™ 555 a superior replacement for Cy3® and Alexa Fluor® 555 dyes. Used for stable signal generation in imaging and flow cytometry, the fluorescence intensity of iFluor™ 555 conjugates are pH-insensitive from pH 4-11. iFluor™ 555 antibody conjugates can be well excited with either Nd:YAG laser (~532 nm), Helium-Neon laser (~543 nm) or Krypton ion laser (~568 nm).

Red Fluorescent Dyes

iFluor™ 568

A Superior Replacement for Alexa Fluor® 568 Dyes

iFluor™ 568 is a bright, orange-red fluorescent dye with an excitation ideally suited for the 568 nm laser line on the Ar-Kr mixed-gas laser and is matched well with RFP filter sets. iFluor™ 568 dyes have spectral characteristics similar to those of Alexa Fluor® 568, and can also be excited by the Helium-Neon laser (~543 nm). iFluor™ 568 dyes are water soluble, and their fluorescence is pH-insensitive from pH 4-10. Compared to Alexa Fluor® 568, iFluor™ 568 dyes are brighter, and have a superior signal-to-background ratios (see Fig. 2.12). Additionally, our iFluor™ 568 streptavidin conjugates provide a high fluorescence intensity and low background as validated in immunofluorescence staining of mammalian cells.

iFluor™ 594

A Superior Replacement for Alexa Fluor® 594 and Texas Red® Dyes

iFluor™ 594 has spectral characteristics similar to Texas Red®, DyLight™ 594 and Alexa Fluor® 594 with an excitation at 592 nm and emission at 614 nm when conjugated to proteins. Compared to Texas Red®, iFluor™ 594 dyes have superior labeling performance with better stability. Our iFluor™ 594 streptavidin conjugates provide a higher signal-to-background ratio as validated in immunofluorescence staining of mammalian cells. Biomolecules conjugated to iFluor™ 594 exhibit little spectral overlap with green-fluorescent conjugates, and can be efficiently excited by either the 568 nm line of Ar-Kr lasers or by the 594 nm line of orange He-Ne lasers. With minimal spectral overlap, iFluor™ 594 dyes are an ideal second color in combination with a green fluorophore such as GFP, FITC, Alexa Fluor® 488 or iFluor™ 488. Our in-house research indicates that the iFluor™ 594-PE conjugates demonstrate better FRET compared to Texas Red®-PE. Under the same test conditions, iFluor™ 594 secondary antibody conjugates gave higher signal-to-background ratios than the corresponding Alexa Fluor® 594-labeled conjugates.

iFluor™ 633

A Superior Replacement for Alexa Fluor® 633 Dyes

iFluor™ 633 dyes are spectrally similar to Alexa Fluor® 633 and DyLight™ 633 dyes.

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Fluorescence emission of iFluor™ 633 dyes is well separated from that of other commonly used red fluorophores, such as TAMRA, Texas Red®, Alexa Fluor® 594, iFluor™ 594 and R-phycoerythrin. iFluor™ 633 dyes can be well excited by the 633 nm red laser in flow cytometers, giving a deep red emission. Compared to Alexa Fluor® 633, the extinction coefficient of iFluor™ 633 is much higher (~250,000 cm-1M-1). Under the same conditions, iFluor™ 633 labeled conjugates gave higher signal-to-background ratios than the corresponding Alexa Fluor® 633-labeled conjugates.

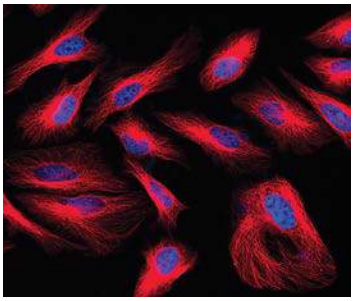

Figure 2.14 Image of HeLa cells. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 633 goat anti-mouse IgG (H&L) (Red, Cat# 16478), and nuclei were stained with DAPI (Blue, Cat# 17507).

iFluor™ 647
A Superior Replacement for Alexa Fluor® 647, DyLight™ 650, and Cy5® Dyes

Cy5® dyes are the preferred dyes for preparing deep red fluorescent bioconjugates. Compared to Cy5® conjugates, the spectra of iFluor™ 647 conjugates are only slightly red-shifted. This slight change in absorption spectrum makes iFluor™ 647 dyes an optimal match to filters designed for Cy5® dyes. In a side-by-side comparison of iFluor™ 647 and Cy5® antibody conjugates, the total fluorescence of iFluor™ 647 labeled antibodies are significantly higher than that of Cy5® conjugates. Unlike Cy5® dyes, iFluor™ 647 dyes have very little change in absorption or fluorescence spectra when conjugated to most proteins and nucleic acids. This yields a greater total fluorescence at the same degree of substitution. iFluor™ 647 dyes are spectrally similar to Alexa Fluor® 647 and DyLight™ 650 dyes making it an excellent replacement for Alexa Fluor® 647 and DyLight™ 650 dyes.

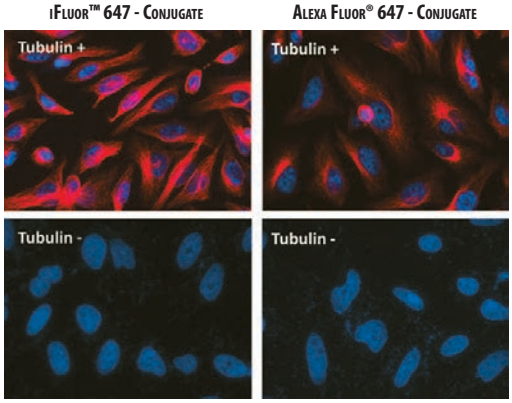

Figure 2.15 Images of HeLa cells. HeLa cells were incubated with (Tubulin+) or without (Tubulin-) mouse anti-tubulin then followed with iFluor™ 647 goat anti-mouse IgG (H&L) (Left, Cat# 16562) or Alexa Fluor® goat anti-mouse IgG (Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17530).

iFluor™ 647 conjugates can be well excited by the Helium-Neon laser (~633 nm), red diode laser (~635 nm) or Krypton ion laser (~647 nm). Under the same conditions, iFluor™ 647 antibody conjugates are brighter, and gave higher signal-to-background ratios than the corresponding Cy5® and Alexa Fluor® 647 antibody conjugates.

iFluor™ 680
A Superior Replacement for and Alexa Fluor® 680, IRDye® 700 and Cy5.5® Dyes

iFluor™ 680 dyes are spectrally similar to Cy5.5®, IRDye® 700, Alexa Fluor® 680 and DyLight™ 680 dyes. Fluorescence emission of iFluor™ 680 dyes are well separated from that of other commonly used red fluorophores, such as TAMRA, R-phycoerythrin, iFluor™ 594, 633 and 647 dyes, making it ideal for three- and four-color labeling. iFluor™ 688 dyes can be effectively excited by the 633 nm red laser in flow cytometers, giving a near infrared emission. iFluor™ 680 dyes are also excellent acceptor dyes for allophycocyanin (APC), further facilitating multicolor flow cytometry analysis. AAT Bioquest offers iFluor™ 688-APC tandem conjugates for flow cytometric applications. iFluor™ 680 dyes are bright and photostable near-IR dyes used for stable signal generation in imaging and flow cytometry. The fluorescence of this long-wavelength iFluor™ 680 dye is not visible to the human eye but is readily detected by most imaging systems.

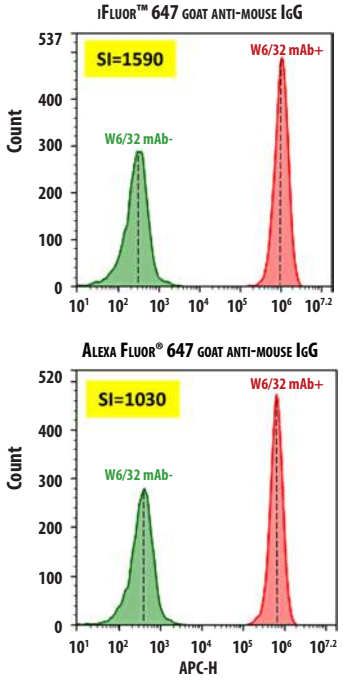

Figure 2.16 HL-60 cells were incubated with (Red, +) or without (Green, -) Anti-human HLA-ABC (W6/32 mAb), followed by iFluor™ 647 goat anti-mouse IgG (H&L) (Cat# 16562) or Alexa Fluor® 647 goat anti-mouse IgG conjugate, respectively. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC channel. The stain index (SI) of each conjugate was calculated. Because iFluor™ 647 conjugates have a higher signal-to-noise ratio, indicative of its larger SI, it is a more sensitive and superior replacement for Alexa Fluor® 647 conjugates.

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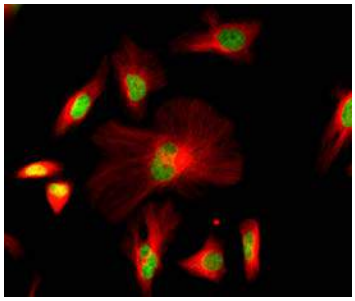

Figure 2.17 Image of HeLa cells. HeLa cells were stained with iFluor™ 680 goat anti-mouse IgG (H&L) (Red, Cat# 16566), and nuclei were stained with Nuclear Green™ DCS1 (Green, Cat# 17550).

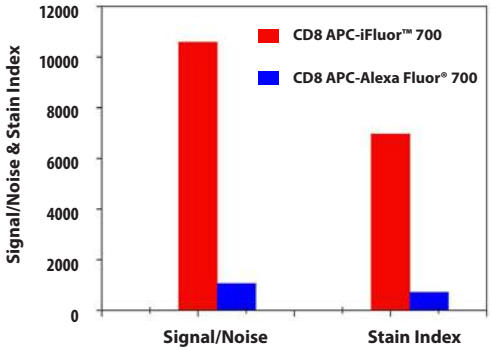

Figure 2.18 Flow cytometric analysis of APC-iFluor ™ 700 (Red Bar, Cat# 2570) or APC-Alexa Fluor® 700 (Blue Bar) anti-human CD8 on human lymphocytes. Whole blood was stained with APC-iFluor ™ 700 or APC-Alexa Fluor® 700 anti-human CD8 and compared to whole blood stained with a APC-iFluor ™ 700 and APC-Alexa Fluor® 700 mouse IgG control. Flow cytometry was performed on a ACEA flow cytometry system.

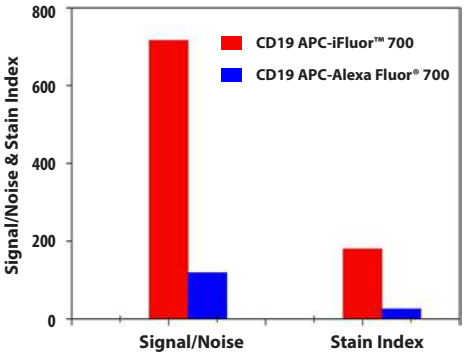

Figure 2.19 Flow cytometric analysis of APC-iFluor ™ 700 (Red Bar, Cat# 2570) or APC-Alexa Fluor® 700 (Blue Bar) anti-human CD19 on human lymphocytes. Whole blood was stained with APC-iFluor ™ 700 or APC-Alexa Fluor® 700 anti-human CD19 and compared to whole blood stained with a APC-iFluor ™ 700 and APC-Alexa Fluor® 700 mouse IgG control. Flow cytometry was performed on a ACEA flow cytometry system.

iFluor™ 700
A Superior Replacement for Alexa Fluor® 700 Dyes

Spectrally similar to Alexa Fluor® 700 dyes, iFluor™ 700 dyes have a fluorescence emission maximum at 710 nm with a fluorescence quantum yield close to 0.2. Compared to Alexa Fluor® 700 dyes, iFluor™ 700 dyes are brighter with a stronger absorption at 633 nm. Fluorescence emission of iFluor™ 700 dyes are well separated from that of other commonly used red fluorophores, such as TAMRA, R-phycoerythrin and iFluor™ 647 dyes, making it ideal for three- and four-color labeling. iFluor™ 700 dyes can be effectively excited by the 633 nm red laser in flow cytometers, giving additional near infrared emission. iFluor™ 700 dyes are also excellent acceptor dyes for allophycocyanin (APC), further facilitating multicolor flow cytometry analysis. AAT Bioquest offers iFluor™ 700-APC tandem conjugates for flow cytometric applications.

iFluor™ 750
A Superior Replacement for Alexa Fluor® 750, DyLight™ 755 and Cy7® Dyes

Spectrally similar to Cy7®, DyLight™ 755 and Alexa Fluor® 750 dyes, iFluor™ 750 dyes have a fluorescence emission maximum at ~780 nm with a fluorescence quantum yield close to 0.1. Compared to Alexa Fluor® 750 dyes, iFluor™ 750 dyes are much brighter with a stronger absorption at 633 nm. Fluorescence emission of iFluor™ 750 dyes are well separated from that of other commonly used red fluorophores, such as TAMRA, R-phycoerythrin and iFluor™ 647 dyes, making it ideal for three- and four-color labeling. iFluor™ 750 dyes can be effectively excited by the 633 nm red laser in flow cytometers, giving additional near infrared emission color. In addition, iFluor™ 750 dyes are also excellent acceptor dyes for allophycocyanin (APC), further facilitating multicolor flow cytometry analysis. AAT Bioquest offers iFluor™ 750-APC tandem conjugates for flow cytometric applications

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Near Infrared and Infrared Fluorescent Labeling Dyes

AAT Bioquest's collections of near infrared and infrared fluorescent labeling dyes are excellent for Western blotting and *in vivo* imaging applications. Near infrared and infrared imaging systems measure the signal generated in a static state eliminating any precautionary steps needed to optimize the detection of dynamic reactions associated with chemiluminescence. Fluorophores detected at the near infrared and infrared wavelengths provide higher sensitivity than those detected in the visible light spectrum. Autofluorescence from membrane surfaces and biomolecules are significantly reduced at longer wavelengths, providing decreased background interference and improving sensitivity. In addition, near infrared and infrared imaging systems have a wider linear detection range, increased stability, and can be optimized for multiplexing. A wide linear detection range in combination with the static state of fluorescence detection allows for all protein concentrations of the sample within the instrument's detectable range to be made visible.

AAT Bioquest offers the largest collection of near infrared and infrared fluorescent labeling dyes optimized for use in multiplexing applications, Western blotting and *in vivo* imaging. AAT Bioquest is the only major commercial source for fluorescent dyes exhibiting a maximum absorption wavelength longer than 820 nm.

iFluor™ 790
An Excellent Replacement for IRDye® 800 and Alexa Fluor® 790 Dyes

Spectrally similar to IRDye® 800 and Alexa Fluor® 790 dyes, iFluor™ 790 dyes might be the brightest fluorophore at 790 nm excitation with fluorescence quantum yield close to 0.1. iFluor™ 790 conjugates have been successfully used for NIR fluorescent probes-based *in vivo* imaging analysis. Its fluorescence emission maximum around 810 nm is well separated from commonly used far-red fluorophores, making this dye family an excellent replacement for IRDye®800 and Alexa Fluor® 790 dyes.

iFluor™ 810
A Superior Infrared Labeling Dye

iFluor™ 810 labeling dyes are designed to label proteins and other biomolecules with infrared fluorescence. iFluor™ 810 conjugates have an excitation and emission in the IR range well separated from commonly used red fluorophores, such as Cy5®, Cy7® or APC, allowing for multicolor analysis. iFluor™ 810 is useful for small animal *in vivo* imaging application or imaging applications requiring IR detections. iFluor™ 810 dyes are available in acid, maleimide and succinimidyl ester labeling chemistries.

iFluor™ 820
A Superior Infrared Labeling Dye

iFluor™ 820 labeling dyes are designed to label proteins and other biomolecules with infrared fluorescence. iFluor™ 820 conjugates have an excitation and emission

Features of Near Infrared and Infrared Fluorescence

Multiplexing:

- **Infrared Fluorescence:** **Yes**

Infrared western blot allows normalization or comparative analysis without stripping and reprobing of blots.

Detection:

- **Infrared Fluorescence:** **Direct**

Signals produced by infrared iFluor™-labeled proteins are directly proportional to the amount of target protein.

Stability:

- **Infrared Fluorescence:** **Months To Years**

Near infrared and infrared iFluor™ dye fluorescent signals are highly stable, so you can store blots and re-image later.

Sensitivity:

- **Infrared Fluorescence:** **Very Sensitive**

Infrared detection is static allowing for a wider linear detection range without any signal loss. Additionally, IR imaging can be performed simultaneously on the same blot for improved detection efficiency.

Time:

- **Infrared Fluorescence:** **Less**

Infrared iFluor™ labeled proteins have a wide dynamic range to save you time by reducing the need for multiple exposures.

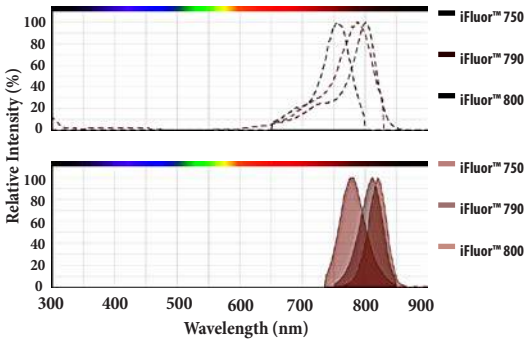

Figure 2.20 Excitation (above) and emission (below) spectra of the near infrared and infrared iFluor™ 750, 790 and 800 dye series.

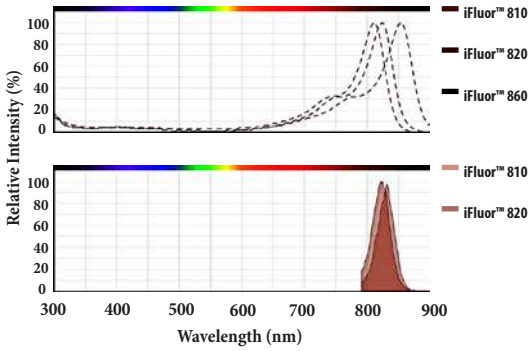

Figure 2.21 Excitation (above) and emission (below) spectra of the infrared iFluor™ 810, 820 and 860 dye series.

in the IR range well separated from commonly used red fluorophores, such as Cy5®, Cy7® or APC, allowing for multicolor analysis. iFluor™ 820 is useful for small animal *in vivo* imaging application or imaging applications requiring IR detections. iFluor™ 820 dyes are available in acid, maleimide and succinimidyl ester labeling chemistries.

iFluor™ 860
A Superior Infrared Labeling Dye

iFluor™ 860 labeling dyes are designed to label proteins and other biomolecules with infrared fluorescence. iFluor™ 860 conjugates have an excitation and emission in the IR range well separated from commonly used red fluorophores, such as Cy5®, Cy7® or APC, allowing for multicolor analysis. iFluor™ 860 is useful for small animal *in vivo* imaging application or imaging applications requiring IR detections. iFluor™ 860 dyes are available in acid, maleimide and succinimidyl ester labeling chemistries.

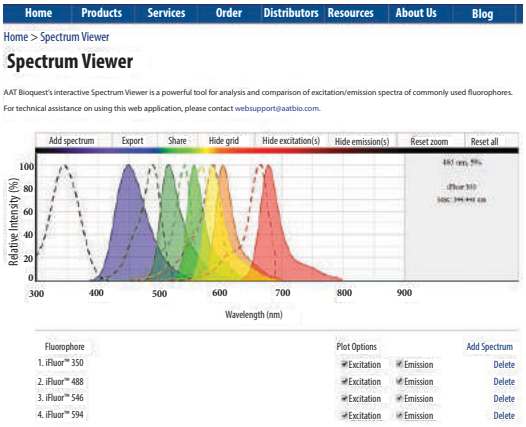

Figure 2.22 Interactive Spectrum Viewer tool available on AAT Bioquest's website.
1. To access the spectrum viewer tool navigate to www.aatbioquest.com
2. Click on the "**Resources"** tab located in the tool bar at the top of the website to activate a drop-down menu, and click Spectrum Viewer.
3. Click on **"Add spectrum"** to access our comprehensive spectral database to add and compare our fluorophores and fluorphores from other companies.
4. Below the spectrum graph you will find all the fluorophores you have added. Here you can hide/show the excitation and emission curves as well as delete any fluorphores from the spectrum you wish to no longer compare.
5. When you are satisfied with you spectrum, save a copy by clicking on the **"Export"** button. The graph will automatically download to your computer as a .PNG file.

AAT Bioquest's Interactive Spectrum Viewer Tool

AAT Bioquest's interactive Spectrum Viewer is a powerful tool that helps scientists easily analyze and compare spectral data. Our Spectrum Viewer includes a comprehensive spectral database consisting of our extensive catalog of fluorophores and fluorophores from other companies. It conveniently provides an instructive one-stop destination for making informed decisions about which products best suit your experimental design and equipment (i.e. lasers and filter sets). Recently, major updates have been made to improve our Spectrum Viewer's overall functionality. Added features include:

- **A "Share" Function** to link graph snapshots to other users
- **An "Export" Function** to save graphs as a .PNG file
- **Advanced Filtering Capabilities** to search for compounds by name, excitation/emission ranges and Stokes shift

Fluorescence Instruments

There are two primary categories of instruments that measure fluorescence. One category consists of spectrofluorometers such as microplate readers, flow cytometers and fluorescence scanners. The other category is comprised of fluorescence microscopes. All fluorescence instruments require the following key elements:

- **Excitation Source**: UV-visible lamps (e.g. LED) or lasers are used to provide the energy required to excite specific fluorophores.
- **Light Collection Optics**: Researches can utilize any ideal combination of optical elements such as lenses, mirrors and filters to develop an efficient imaging system suitable for their experimental needs.
- **Detection, Amplification and Digitization**: Photomultiplier tubes (PMT) or a charged-coupled device (CCD) are used for the detection and quantification of emitted light by converting emitted fluorescent light into measurable electrical energy.

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Product Ordering Information For iFluor™ Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
1070	iFluor™ 350 amine	1 mg	346	449
1080	iFluor™ 350 hydrazide	1 mg	346	449
1060	iFluor™ 350 maleimide	1 mg	346	443
1020	iFluor™ 350 succinimidyl ester	1 mg	346	443
1071	iFluor™ 405 amine	1 mg	401	420
1081	iFluor™ 405 hydrazide	1 mg	401	420
1021	iFluor™ 405 succinimidyl ester	1 mg	401	420
1072	iFluor™ 488 amine	1 mg	491	514
1000	iFluor™ 488 azide	1 mg	491	518
1082	iFluor™ 488 hydrazide	1 mg	491	514
1062	iFluor™ 488 maleimide	1 mg	491	518
1023	iFluor™ 488 succinimidyl ester	1 mg	491	518
11060	iFluor™ 488 tyramide	1 mg	491	514
1024	iFluor™ 514 succinimidyl ester	1 mg	518	542
1025	iFluor™ 532 succinimidyl ester	1 mg	531	556
1048	iFluor™ 546 succinimidyl ester	1 mg	541	557
1092	iFluor™ 555 alkyne	1 mg	555	565
1073	iFluor™ 555 amine	1 mg	555	565
1093	iFluor™ 555 azide	1 mg	555	565
1083	iFluor™ 555 hydrazide	1 mg	559	569
1063	iFluor™ 555 maleimide	1 mg	555	565
1028	iFluor™ 555 succinimidyl ester	1 mg	555	565
1049	iFluor™ 568 succinimidyl ester	1 mg	568	587
1029	iFluor™ 594 succinimidyl ester	1 mg	594	614
1038	iFluor™ 610 succinimidyl ester	1 mg	605	627
1030	iFluor™ 633 succinimidyl ester	1 mg	638	655
1090	iFluor™ 647 alkyne	1 mg	649	664
1074	iFluor™ 647 amine	1 mg	649	664
1091	iFluor™ 647 azide	1 mg	649	664
1085	iFluor™ 647 hydrazide	1 mg	649	664
1065	iFluor™ 647 maleimide	1 mg	649	664
1031	iFluor™ 647 succinimidyl ester	1 mg	649	664
1075	iFluor™ 660 amine	1 mg	662	678
1032	iFluor™ 660 succinimidyl ester	1 mg	662	678
1076	iFluor™ 680 amine	1 mg	676	695
1086	iFluor™ 680 hydrazide	1 mg	676	695
1066	iFluor™ 680 maleimide	1 mg	676	695
1035	iFluor™ 680 succinimidyl ester	1 mg	676	695
1077	iFluor™ 700 amine	1 mg	685	710
1087	iFluor™ 700 hydrazide	1 mg	685	710
1067	iFluor™ 700 maleimide	1 mg	685	710
1036	iFluor™ 700 succinimidyl ester	1 mg	685	710

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Product Ordering Information For iFlour™ Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
1078	iFluor™ 710 amine	1 mg	712	736
1045	iFluor™ 710 succinimidyl ester	1 mg	712	736
1079	iFluor™ 750 amine	1 mg	749	775
1088	iFluor™ 750 hydrazide	1 mg	749	775
1068	iFluor™ 750 maleimide	1 mg	749	775
1037	iFluor™ 750 succinimidyl ester	1 mg	749	775
1360	iFluor™ 790 acid	5 mg	782	811
1362	iFluor™ 790 amine	1 mg	782	811
1364	iFluor™ 790 hydrazide	1 mg	782	811
1366	iFluor™ 790 maleimide	1 mg	782	811
1368	iFluor™ 790 succinimidyl ester	1 mg	782	811
1375	iFluor™ 800 acid	1 mg	801	820
1378	iFluor™ 800 maleimide	1 mg	801	820
1379	iFluor™ 800 succinimidyl ester	1 mg	801	820
1385	iFluor™ 810 acid	1 mg	811	825
1388	iFluor™ 810 maleimide	1 mg	811	825
1389	iFluor™ 810 succinimidyl ester	1 mg	811	825
1395	iFluor™ 820 acid	1 mg	824	831
1398	iFluor™ 820 maleimide	1 mg	824	831
1399	iFluor™ 820 succinimidyl ester	1 mg	824	831
1405	iFluor™ 860 acid	1 mg	863	868
1408	iFluor™ 860 maleimide	1 mg	863	868
1409	iFluor™ 860 succinimidyl ester	1 mg	863	868

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mFluor™ Fluorescent Labeling Dyes

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Principle Excitation Lines (nm) of Common Light Sources Used in Flow Cytometers	
Violet Laser	405 nm
Blue Laser	488 nm
Green Laser	532 nm
Yellow-Green Laser	561 nm
Red Laser	633 nm

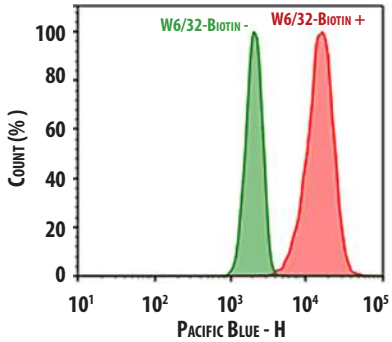

Figure 3.1 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1µg/ml Anti-Human HLA-ABC-Biotin and then followed by mFluor™ Violet 450-streptavidin conjugate (Cat#16930). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the Pacific Blue channel.

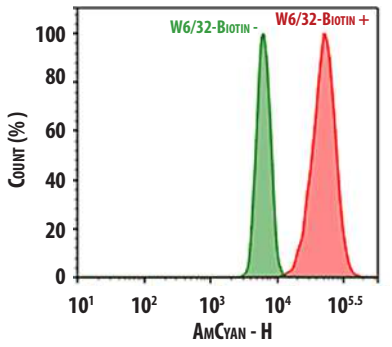

Figure 3.2 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1µg/ml Anti-Human HLA-ABC-Biotin then followed by mFluor™ Violet 510-streptavidin conjugate (Cat#16931). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the AmCyan channel.

Here at AAT Bioquest, we are rapidly expanding and improving our product lines to better meet constantly changing research needs. Our mFluor™ dyes are a result of recent research and development efforts focusing on resolving limitations associated with existing fluorescent labeling technologies. mFluor™ dyes are a series of excellent fluorescent labeling dyes that span the full UV-visible spectrum. All the mFluor™ dyes are designed to be maximally excited by one of the major light sources in flow cytometers such as the violet laser at 405 nm or blue laser at 488 nm. mFluor™ dyes are excellent alternatives to the phycoprotein-based tandems that are quite difficult to couple to an antibody or other biomolecules.

mFluor™ dyes have the following features:

- mFluor™ dyes are robust, more photostable than phycoprotein tandems, highly fluorescent over a broad pH range and available in a variety of reactive forms such as amines and thiols.
- mFluor™ dyes can be easily conjugated to proteins and other biomolecules, providing higher conjugation yields than tandems.
- mFluor™ dyes conjugates can be maximally excited by one of the major light sources used in flow cytometers.

Blue Fluorescent Dyes

mFluor™ Violet 450

An Excellent Replacement for Pacific Blue® Dyes

mFluor™ Violet 450 dyes are an excellent replacement for Pacific Blue® dyes because of their nearly identical spectral properties. mFluor™ Violet 450 dyes have improved water-solubility compared to Pacific Blue® dyes. Protein conjugates prepared with mFluor™ Violet 450 are bright, and are excellent violet laser reagents for flow cytometry research.

Green Fluorescent Dyes

mFluor™ Violet 510

An Excellent Replacement for AmCyan

mFluor™ Violet 510 dyes are an excellent alternative for AmCyan because of their nearly identical spectral properties. mFluor™ Violet 510 dyes are water-soluble, and protein conjugates prepared with mFluor™ Violet 510 dyes are well excited at 405 nm giving a green fluorescent signal (compatible with FITC filter). mFluor™ Violet 510 dyes and conjugates are excellent violet laser reagents for flow cytometry research.

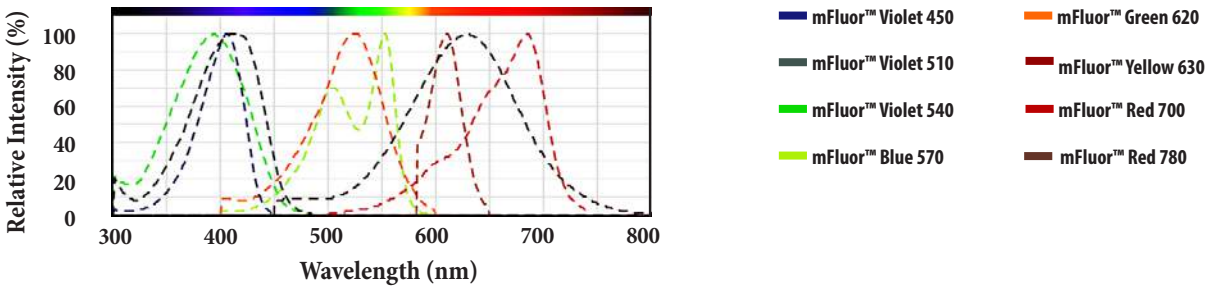

Figure 3.3 Excitation Spectrum of mFluor™ Labeling Dyes

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Orange Fluorescent Dyes

mFluor™ Violet 540

An Excellent Replacement for Pacific Orange® and Krome Orange™ Dyes

mFluor™ Violet 540 dyes are an excellent replacement for Pacific Orange® and Krome Orange™ dyes because of their equivalent spectral properties. mFluor™ Violet 540 dyes are water-soluble, and some of the protein conjugates prepared with mFluor™ Violet 540 dyes are brighter than those prepared with Pacific Orange® and Krome Orange™. mFluor™ Violet 540 dyes and conjugates are excellent violet laser reagents for flow cytometry research.

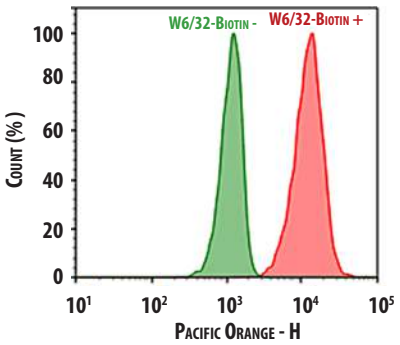


Figure 3.4 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1 µg/ml Anti-Human HLA-ABC-Biotin and then followed by mFluor™ Violet 540-streptavidin conjugate (Cat#16932). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the Pacific Orange channel.

Red Fluorescent Dyes

mFluor™ Blue 570

An Excellent Replacement for R-Phycoerythrin (PE)

mFluor™ Blue 570 dyes have spectral properties equivalent to those of PE conjugates making it an excellent alternative for PE. mFluor™ Blue 570 dyes are water-soluble, and protein conjugates prepared with mFluor™ Blue 570 dyes are well excited at 488 nm giving a red fluorescent signal (compatible with TRITC filter). mFluor™ Blue 570 dye and protein conjugates are excellent blue laser reagents for flow cytometry research. Compared to PE conjugates which experience rapid photobleaching, mFluor™ Blue 570 dyes are much more photostable, making them readily available for fluorescence imaging applications.

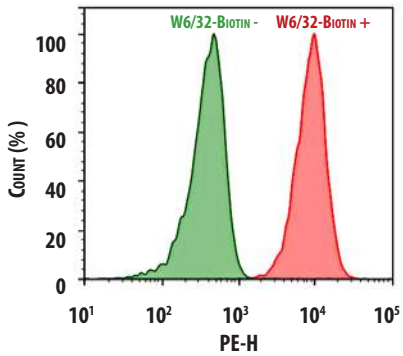


Figure 3.5 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1 µg/ml Anti-Human HLA-ABC-Biotin and then followed by mFluor™ Blue 570-streptavidin conjugate (Cat#16935).

mFluor™ Green 620

A Unique Dye that is Well Excited by the Green Laser at 532 nm

mFluor™ Green 620 is a unique red fluorescent dye that is well excited by the green laser at 532 nm. mFluor™ Green 620 dye is water-soluble, and protein conjugates prepared with mFluor™ Green 620 dyes are bright with a Stokes Shift of ~80 nm. mFluor™ Green 620 dyes and conjugates are excellent green laser reagents for both flow cytometry research and fluorescence imaging applications.

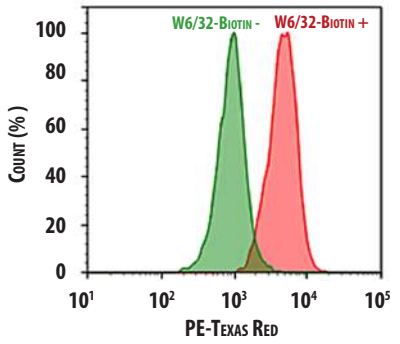
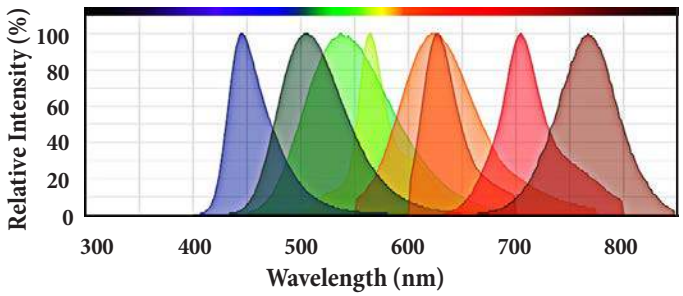


Figure 3.6 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1 µg/ml Anti-Human HLA-ABC-Biotin and then followed by mFluor™ Green 620-streptavidin conjugate (Cat#16938).

mFluor™ Yellow 630

A Unique Dye that is Well Excited by the Green-Yellow Laser at 561 nm

mFluor™ Yellow 630 is a unique red fluorescent dye that is well excited by the green-yellow laser at 561 nm. mFluor™ Yellow 630 dye is water-soluble, and protein conjugates prepared with mFluor™ Yellow 630 dyes are bright. mFluor™ Yellow 630



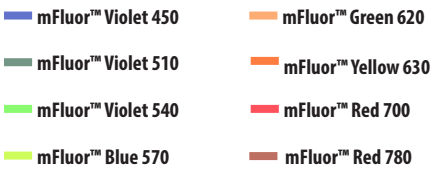


Figure 3.7 Emission Spectrum of mFluor™ Labeling Dyes

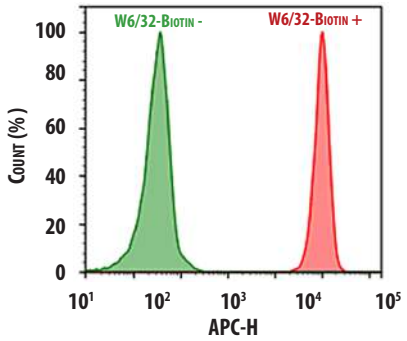


Figure 3.8 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1 µg/ml Anti-Human HLA-ABC-Biotin and then followed by mFluor™Red 700-streptavidin conjugate (Cat#16946). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC channel.

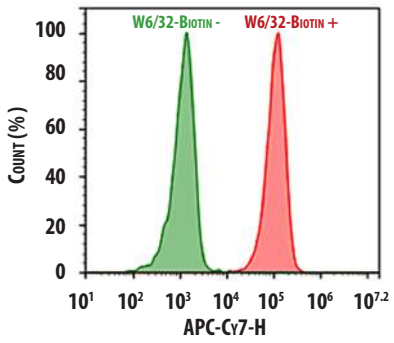


Figure 3.9 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1 µg/ml Anti-Human HLA-ABC-Biotin and then followed by mFluor™ Red 780-streptavidin conjugate (Cat#16948). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC-Cy7 channel.

dyes and conjugates are excellent yellow laser reagents for both flow cytometry research and fluorescence imaging applications.

mFluor™ Red 700
An Excellent Replacement for APC-Alexa Fluor® 700 Tandems

mFluor™ Red 700 dyes are an excellent alternative for APC-Alexa Fluor® 700 tandems since they have the spectral properties equivalent to those of APC-Alexa Fluor® 700 conjugates. mFluor™ Red 700 dyes are water-soluble, and the protein conjugates prepared with mFluor™ Red 700 dyes are well excited at 633 nm to give red fluorescence (compatible with Cy5.5® filter). mFluor™ Red 700 dyes and conjugates are excellent red laser reagents for flow cytometry research. Compared to APC-Alexa Fluor® 700 dyes, mFluor™ Red 700 dyes are much more photostable than the spectrally similar APC tandems, making them readily available for fluorescence imaging applications while it is very difficult to use the APC-Alexa Fluor® 700 conjugates for fluorescence imaging applications due to the rapid photobleaching of the APC tandems (such as APC-Alexa Fluor® 700).

mFluor™ Red 780
An Excellent Replacement for APC-Alexa Fluor® 750 Tandems

mFluor™ Red 780 dyes are an excellent alternative for APC-Alexa Fluor® 750 tandems because they have equivalent spectral properties. Compared to APC-Alexa Fluor® 750 tandems, mFluor™ Red 780 dyes are much more photostable, making them readily available for fluorescence imaging applications while it is very difficult to use the APC-Alexa Fluor® 750 conjugates for fluorescence imaging applications due to the rapid photobleaching of APC-Alexa Fluor® 750 tandems. mFluor™ Red 780 dyes are water-soluble, and protein conjugates prepared with mFluor™ Red 780 dyes are well excited at 633 nm giving a red fluorescent signal (compatible with Cy7® filter). mFluor™ Red 780 dyes and conjugates are excellent red laser reagents for flow cytometry research.

Product Ordering Information For mFluor™ Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
1140	mFluor™ Violet 450 acid	5 mg	403	454
1141	mFluor™ Violet 510 acid	5 mg	414	508
1142	mFluor™ Violet 540 acid	5 mg	405	537
1143	mFluor™ Blue 570 acid	5 mg	553	570
1144	mFluor™ Green 620 acid	5 mg	523	617
1145	mFluor™ Yellow 630 acid	5 mg	611	630
1146	mFluor™ Red 700 acid	5 mg	657	700
1147	mFluor™ Red 780 acid	5 mg	629	780
1150	mFluor™ Violet 450 SE	1 mg	403	454
1151	mFluor™ Violet 510 SE	1 mg	414	508
1152	mFluor™ Violet 540 SE	1 mg	405	537
1160	mFluor™ Blue 570 SE	1 mg	553	570
1165	mFluor™ Green 620 SE	1 mg	523	617
1170	mFluor™ Yellow 630 SE	1 mg	611	630
1190	mFluor™ Red 700 SE	1 mg	657	700
1191	mFluor™ Red 780 SE	1 mg	629	780
1210	mFluor™ Red 780 amine	1 mg	629	780

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trFluor™
Fluorescent Labeling Dyes

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Features of trFluor™ Dyes

- **trFluor™ dyes** are available in a variety of reactive forms such as amine-reactive (SE) and thiol reactive (maleimide).
- **trFluor™ dyes** are much easier to be conjugated to proteins and other biomolecules, giving much higher conjugation yield than other europium and terbium dyes.
- **trFluor™ conjugates** are maximally excited by the common light sources at ~350 nm.
- **To maximize** its TR-FRET potential, trFluor™ Eu dye is optimized to pair with APC, iFluor™ 647, TF5, Cy5®, DyLight™ 650 or Alexa Fluor® 647.
- **To maximize** its TR-FRET potential, trFluor™ Tb dye is optimized to pair with FITC, iFluor™ 488, TF2, DyLight™ 488 or Alexa Fluor® 488.
- **No fluoride addition is required.**
- **No enhancing solution is required.**

Table 4.1 Typical acceptors for the time-resolved luminescent probes

trFluor™ Donors	Recommended Acceptors
trFluor™ Eu	iFluor™ 647, TF5, APC
trFluor™ Tb	iFluor™ 488, TF2, FITC

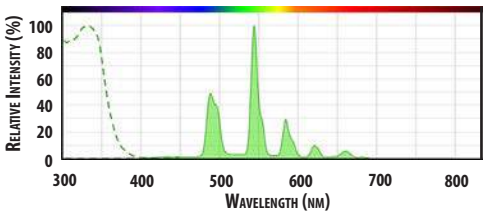

Figure 4.1 Excitation and emission spectra of trFluor™ Tb.

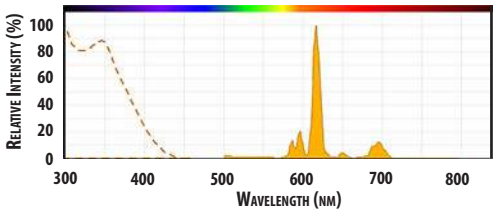

Figure 4.2 Excitation and emission spectra of trFluor™ Eu.

Many biological compounds present in cells, serum or other biological fluids are naturally fluorescent. Therefore the use of conventional, short-lived fluorophores leads to serious limitations in assay sensitivity due to the high background interference caused by the autofluorescence of these biological molecules. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes short-lived fluorescence interferences. Our trFluor™ probes enable time-resolved fluorimetry (TRF) for assays requiring high sensitivity. These trFluor™ probes have large Stokes shifts and extremely long emission half-lives when compared to traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to other TRF compounds, our trFluor™ probes have relatively high stability, high emission yield and the versatility to be linked to biomolecules. Moreover, our trFluor™ Eu probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies.

trFluor™ Tb

An Excellent Building Block for Developing TR-FRET Assays

Our trFluor™ Tb probes enable TRF for assays that require high sensitivity. The trFluor™ Tb dyes have a large Stokes shifts and extremely long emission half-lives when compared to traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to other TRF compounds, our trFluor™ Tb probes have relatively high stability, high emission yield and a higher conjugation yield when linked to biomolecules. Moreover, our trFluor™ Tb probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies. To maximize the TR-FRET potential, trFluor™ Tb dyes are optimized to pair with FITC, iFluor™ 488, TF2, DyLight™ 488 and Alexa Fluor® 488.

trFluor™ Eu

An Excellent Building Block for TR-FRET Assays

Our trFluor™ Eu probes enable TRF for assays that require high sensitivity. Compared to traditional fluorophores such as Alexa Fluor® or cyanine dyes, trFluor™ Eu dyes have a large Stokes shifts and extremely long emission half-lives. When compared to the other TRF compounds, our trFluor™ Eu probes have relatively high stability, high emission yield and the ability to be linked to biomolecules with higher conjugation yield. Moreover, our trFluor™ Eu probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies. To maximize the TR-FRET potential, trFluor™ Eu dyes are optimized to pair with APC, iFluor™ 647, TF5, Cy5®, DyLight™ 650 and Alexa Fluor® 647.

Product Ordering Information For trFluor™ Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
1433	trFluor™ Eu succinimidyl ester	1 mg	346	617
1434	trFluor™ Eu maleimide	100 µg	346	617
1443	trFluor™ Tb succinimidyl ester	1 mg	330	544
1444	trFluor™ Tb maleimide	100 µg	330	544

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Classic Fluorescent Labeling Dyes

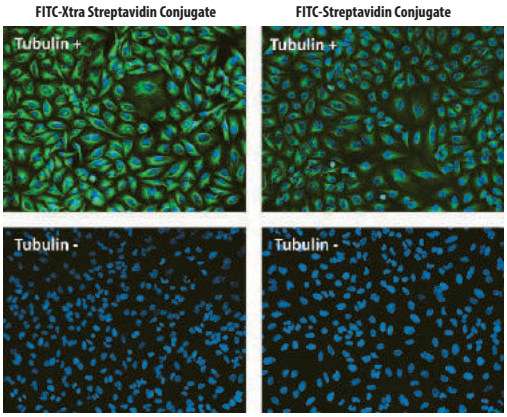

Figure 5.1 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin and biotin goat anti-mouse IgG followed by FITC-Xtra streptavidin conjugate (Green, Left, Cat# 135) or FITC-streptavidin conjugate (Green, Right, Cat# 16910), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17530).

Table 5.1 Comparison of 5-FITC and FITC-xtra.

Dye	5-FITC	FITC-xtra
Optimal pH range for fluorescence detection	>9	4-11
Brightness	Moderate	High
Signal/background ratio	Moderate	High
Photostability	Poor	Excellent
Optimal conjugation pH	>10	8.5-9.5
Conjugation yield	Moderate	High

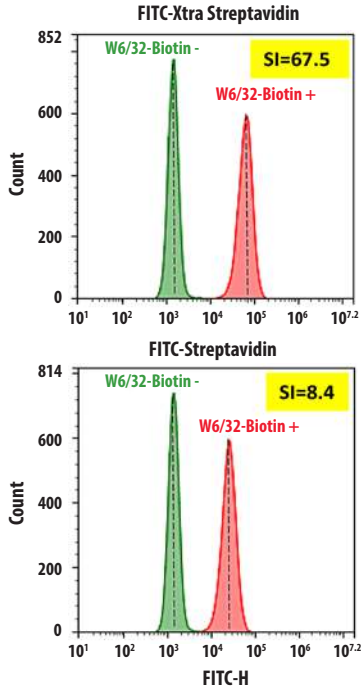

Figure 5.2 HL-60 cells were incubated with (Red, +) or without (Green, -) mouse anti-HLA-ABC (W6/32 mAb) and biotin goat anti-mouse IgG followed by FITC-Xtra streptavidin conjugate (Cat# 135) or FITC-streptavidin conjugate (Cat# 16910), respectively. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in FITC channel. The stain index (SI) of each conjugate was calculated.

Classic reactive fluorescent labeling dyes are useful for conventional applications such as imaging and flow cytometry. They are also suitable for distinct experimental designs involving the exploitation of FITC's high rate of photobleaching or pH sensitivity. These classic reactive fluorescent dyes are widely used to modify proteins, nucleic acids and other biological molecules into fluorescent probes enabling researchers to detect particular components of complex biomolecular assembles with exquisite sensitivity and selectivity. AAT Bioquest offers a full spectrum of classic fluorescent labeling dyes available in various labeling chemistries suitable for any research application. Our classic labeling dyes are designed to react with alkyne, amine, azide, carbonyl, carboxy and thiol moieties.

Traditional fluorescent labeling dyes and other classic labeling dyes now have superior iFluor™ labeling dye replacements. These alternatives have improved fluorescent intensity, greater photostability and a broader optimal pH range.

Alexa Fluor® Fluorescent Labeling Dyes

AAT Bioquest offers a small collection of Alexa Fluor® reactive labeling dyes designed to target amine and thiol moieties for labeling biopolymers. Alexa Fluor® reactive labeling dyes can be used to modify proteins such as antibodies, nucleic acids and other biomolecules used in immunofluorescence assays, cellular imaging and flow cytometry.

FITC Labeling Dyes

Although FITC is still the most popular fluorescent labeling dye for preparing green fluorescent bioconjugates, it has many limitations such as severe photobleaching for microscope imaging and pH-sensitive fluorescence. Protein conjugates prepared with FITC-xtra are far superior compared to the corresponding FITC conjugates. FITC-xtra conjugates are significantly brighter than FITC conjugates and are much more photostable. Additionally, the fluorescence of FITC-xtra is not affected by pH (4-10). This pH insensitivity is a major improvement over FITC, which emits its maximum fluorescence only at pH above 9.

FITC-xtra has spectral properties almost identical FITC. Like 5-FITC, FITC-xtra antibody conjugates have an excitation ideally suited to the 488 nm laser line, making them alternatives to the corresponding FITC-labeled antibody conjugates. Under the same conditions, FITC-xtra antibody conjugates gave much higher signal-to-background ratios than the corresponding FITC-labeled conjugates. FITC-xtra also gave a higher conjugation yield under mild conjugation conditions compared to 5-FITC.

Texas Red® and California Red™ Dyes

Although Texas Red® is the most popular labeling reagent of sulfonyl chloride, it is quite unstable in water. Even in anhydrous DMF, Texas Red® tends to give a very complicated reaction mixture. It reacts with thiols, alcohols, phenols, aliphatic amines and aromatic amines indiscriminatingly. California Red™ is a succinimidyl ester with spectral properties identical to those of Texas Red®. California Red™ is a superior

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replacement for Texas Red® because it only reacts with aliphatic amines such as amino acids, peptides and proteins, resulting in an extremely stable bright red fluorescent conjugate.

Compared to Texas Red®, California Red™ has a much higher labeling efficiency, and more importantly the resulted conjugates often offer higher signal-to-background ratios for fluorescence imaging or flow cytometry applications. We strongly recommend replacing Texas Red® with California Red™ for labeling proteins, peptides and oligonucleotides. Under the same conditions, California Red™-labeled secondary antibody conjugates give much higher signal-to-background ratios than the corresponding Texas Red®-labeled conjugates.

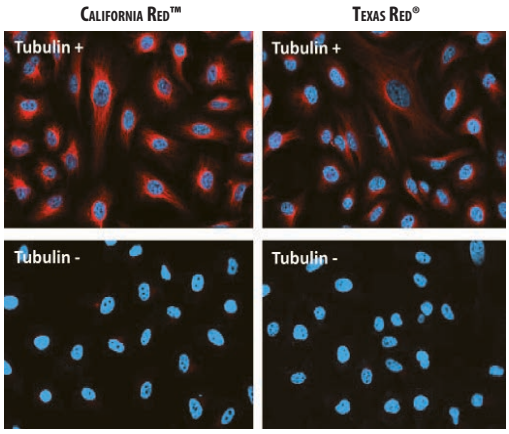

Figure 5.3 HeLa cells were incubated with (Tubulin+) or without (Tubulin-) mouse anti-tubulin followed by goat anti-mouse IgG conjugated with California Red™ (Red, Left) or Texas Red® (Red, Right), respectively. Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17530).

Cyanine Labeling Dyes

A variety of cyanine dyes have been used to label biological molecules such as peptides, proteins and oligonucleotides for fluorescence imaging and other fluorescence-based biochemical assays and applications. AAT Bioquest offers a comprehensive line of cyanine dyes that are readily available in various forms of labeling chemistries and have enhanced fluorescence upon binding to proteins.

Cyanine 3 (Cy3®) dyes are suited for immunocytochemistry applications and commonly used to label nucleic acids. They can be excited by the 532 nm laser line with a maximum excitation at 555 nm and a maximum emission at 565 nm, and can be visualized with TRITC (tetramethylrhodamine) filter sets. Cyanine 3.5 (Cy3.5®) dyes are well suited for fluorescence resonance energy transfer (FRET) and time resolved-fluorescence resonance energy transfer (TR-FRET) applications. They have a maximum excitation at 581 nm and a maximum emission at 596 nm.

Cyanine 5 (Cy5®) dyes are one of the most common and versatile types of red fluorophores. Cyanine 5 dyes can tolerate a pH range of 3-10 making them excellent for use in a variety of applications at biologically relevant pHs. Cyanine 5 dyes are DMSO tolerant and photostable enabling for transfer from storage to assay without any loss in performance. Its aqueous solubility eliminates the need for organic solvents in the assay buffers. Cyanine 5 dyes can be excited by the 633 nm laser line with a maximum excitation at 649 nm and a maximum emission at 665 nm, and can be visualized with Cy5® filter set.

Cyanine 5.5 (Cy5.5®) is far-red emitting dye used for labeling oligonucleotides and are suitable for real time-PCR applications. Cyanine 5.5 dyes have a maximum excitation at 678 nm and a maximum emission at 701 nm.

Cyanine 7 (Cy7®) dyes are one of the most common near infrared fluorophores used in *in vivo* imaging applications due to its low background and high signal-to-noise ratio. Cyanine 7 dyes have a maximum excitation at 749 nm and a maximum emission at 776 nm.

Table 5.2 Comparison of Texas Red® and California Red™.

Dye	Texas Red®	California Red™
Brightness	Moderate	High
Signal/background ratio	Moderate	High
Solvent for preparing stock solution	DMF*	DMSO, DMF or ethanol
Optimal conjugation pH	>10	8.5-9.5
Conjugation yield	Moderate	High
Conjugation yield	Moderate	High

***Neither DMSO nor ethanol can be used for making the stock solution of Texas Red® due to its strong reactivity with these solvents.**

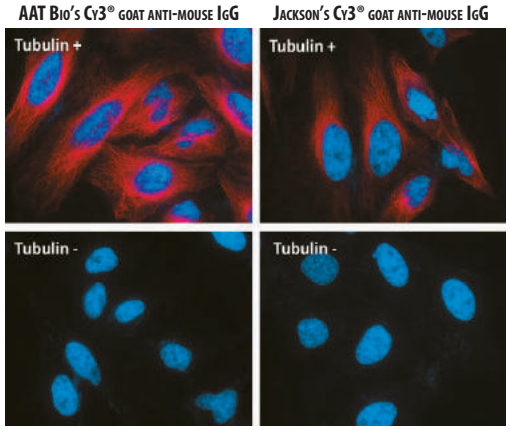

Figure 5.4 Image comparison of HeLa cells. HeLa cells were incubated with (Tubulin +) or without (Tubulin -) mouse anti-tubulin IgG followed by AAT's Cy3® goat anti-mouse IgG (H&L) (Red, Left, Cat# 16862) or Jackson's goat anti-mouse IgG conjugated withCy3® (Right). Cell nuclei were stained with Hoechst 33342 (Blue, Cat# 17530).

Product Ordering Information For Near Infrared, Infrared and Classic Fluorescent Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
423	5(6)-TAMRA C6 maleimide	5 mg	544	575
412	5(6)-TAMRA Maleimide [Tetramethylrhodamine-5-(and-6)-maleimide] *Mixed isomers*	5 mg	540	567
410	5(6)-TRITC [Tetramethylrhodamine-5-(and-6)-isothiocyanate] *CAS 95197-95-8*	5 mg	543	571
409	5(6)-TRITC [Tetramethylrhodamine-5-(and-6)-isothiocyanate] *CAS 95197-95-8*	25 mg	543	571
122	5-FITC [Fluorescein-5-isothiocyanate] *CAS 3326-32-7*	10 g	492	515
121	5-FITC [Fluorescein-5-isothiocyanate] *CAS 3326-32-7*	1 g	492	515
120	5-FITC [Fluorescein-5-isothiocyanate] *CAS 3326-32-7*	100 mg	492	515
708	5-OG488 acid [equivalent to Oregon Green® 488 carboxylic acid, 5-isomer]	25 mg	496	524
710	5-OG488 succinimidyl ester [equivalent to Oregon Green® 488 carboxylic acid, succinimidyl ester, 5-isomer]	5 mg	496	524
424	5-TAMRA C6 maleimide	5 mg	544	575
421	5-TAMRA Maleimide [Tetramethylrhodamine-5-maleimide] *CAS 174568-67-3*	1 mg	540	567
416	5-TRITC [Tetramethylrhodamine-5-isothiocyanate] *CAS 80724-19-2*	5 mg	543	571
415	5-TRITC [Tetramethylrhodamine-5-isothiocyanate] *CAS 80724-19-2*	1 mg	543	571
712	6-OG488 acid [equivalent to Oregon Green® 488 carboxylic acid, 6-isomer]	25 mg	496	524
711	6-OG488 succinimidyl ester [equivalent to Oregon Green® 488 carboxylic acid, succinimidyl ester, 6-isomer]	5 mg	496	524
426	6-ROX C2 Maleimide	1 mg	575	602
425	6-TAMRA C6 maleimide	5 mg	544	575
419	6-TAMRA Maleimide [Tetramethylrhodamine-6-maleimide] *CAS 174568-68-4*	1 mg	540	567
418	6-TRITC [Tetramethylrhodamine-6-isothiocyanate] *CAS 80724-20-5*	5 mg	544	572
417	6-TRITC [Tetramethylrhodamine-6-isothiocyanate] *CAS 80724-20-5*	1 mg	544	572
1870	AF350 C5 Maleimide	1 mg	346	445
1800	AF350 NHS Ester (Succinimidyl Ester)	1 mg	346	445
1803	AF405 NHS Ester (Succinimidyl Ester)	1 mg	400	424
1878	AF488 C5 Maleimide	1 mg	494	517
1812	AF488 NHS Ester (Succinimidyl Ester)	1 mg	494	517
1885	AF532 C5 Maleimide	1 mg	530	555
1819	AF532 NHS Ester (Succinimidyl Ester)	1 mg	530	555
1891	AF594 C5 Maleimide	1 mg	590	617
1830	AF594 NHS Ester (Succinimidyl Ester)	1 mg	590	617
501	AMCA acid *CAS#: 106562-32-7*	25 mg	353	455
503	AMCA C2 Maleimide	5 mg	353	455
504	AMCA Ethylenediamine	5 mg	353	455
502	AMCA, succinimidyl ester	10 mg	353	455
479	California Red™ SE	1 mg	583	603
473	California Red™ SE	5 mg	583	603
190	Cy3NS acid *CAS 1032678-01-5*	100 mg	549	565
191	Cy3NS succinimidyl ester	25 mg	549	565
199	Cy5.5NS succinimidyl ester	10 mg	678	701
196	Cy5.5NS, acid	25 mg	678	701
194	Cy5NS acid	100 mg	644	665

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Product Ordering Information For Near Infrared, Infrared and Classic Fluorescent Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
195	Cy5NS succinimidyl ester	25 mg	644	665
197	Cy7NS acid	100 mg	750	780
198	Cy7NS succinimidyl ester	25 mg	750	780
144	Cyanine 3 alkyne [equivalent to Cy3® alkyne]	1 mg	555	565
145	Cyanine 3 amine [equivalent to Cy3® amine]	1 mg	555	565
143	Cyanine 3 azide [equivalent to Cy3® azide]	1 mg	555	565
137	Cyanine 3 bisacid [equivalent to Cy3® bisacid]	5 mg	555	565
138	Cyanine 3 bissuccinimidyl ester [equivalent to Cy3® bisNHS ester]	1 mg	555	565
270	Cyanine 3 bissuccinimidyl ester, potassium salt [same as GE Cy3® bisNHS ester]	1 mg	555	565
146	Cyanine 3 hydrazide [equivalent to Cy3® hydrazide]	1 mg	555	565
142	Cyanine 3 maleimide [equivalent to Cy3® maleimide]	1 mg	555	565
140	Cyanine 3 monoacid [equivalent to Cy3® acid]	5 mg	555	565
271	Cyanine 3 monosuccinimidyl ester, potassium salt [same as GE Cy3® NHS ester]	1 mg	555	565
141	Cyanine 3 monosuccinimidyl ester [equivalent to Cy3® NHS ester]	1 mg	555	565
147	Cyanine 3.5 acid [equivalent to Cy3.5® acid]	5 mg	581	596
139	Cyanine 3.5 amine [equivalent to Cy3.5® amine]	1 mg	581	596
149	Cyanine 3.5 Maleimide [equivalent to Cy3.5® Maleimide]	1 mg	581	596
148	Cyanine 3.5 monosuccinimidyl ester [equivalent to Cy3.5® NHS ester]	1 mg	581	596
275	Cyanine 3.5 monosuccinimidyl ester, potassium salt [same as GE Cy3.5® NHS ester]	1 mg	581	596
154	Cyanine 5 alkyne [equivalent to Cy5® alkyne]	1 mg	649	665
155	Cyanine 5 amine [equivalent to Cy5® amine]	1 mg	649	665
153	Cyanine 5 azide [equivalent to Cy5® azide]	1 mg	649	665
159	Cyanine 5 bisacid [equivalent to Cy5® bisacid]	5 mg	649	665
157	Cyanine 5 bissuccinimidyl ester [equivalent to Cy5® bisNHS ester]	1 mg	649	665
282	Cyanine 5 bissuccinimidyl ester, potassium salt [same as GE Cy5® bisNHS ester]	1 mg	649	665
156	Cyanine 5 hydrazide [equivalent to Cy5® hydrazide]	1 mg	649	665
152	Cyanine 5 maleimide [equivalent to Cy5® maleimide]	1 mg	649	665
150	Cyanine 5 monoacid [equivalent to Cy5® acid]	5 mg	649	665
151	Cyanine 5 monosuccinimidyl ester [equivalent to Cy5® NHS ester]	1 mg	649	665
280	Cyanine 5 monosuccinimidyl ester, potassium salt [same as GE Cy5® NHS ester]	1 mg	649	665
179	Cyanine 5.5 alkyne [equivalent to Cy5.5® alkyne]	1 mg	678	701
176	Cyanine 5.5 amine [equivalent to Cy5.5® amine]	1 mg	678	701
178	Cyanine 5.5 azide [equivalent to Cy5.5® azide]	1 mg	678	701
172	Cyanine 5.5 bisacid [equivalent to Cy5.5® bisacid]	5 mg	678	701
158	Cyanine 5.5 bissuccinimidyl ester [equivalent to Cy5.5® bisNHS ester]	1 mg	678	701
285	Cyanine 5.5 bissuccinimidyl ester, potassium salt [same as GE Cy5.5® bisNHS ester]	1 mg	678	701
177	Cyanine 5.5 hydrazide [equivalent to Cy5.5® hydrazide]	1 mg	678	701
175	Cyanine 5.5 maleimide [equivalent to Cy5.5® maleimide]	1 mg	678	701
173	Cyanine 5.5 monoacid [equivalent to Cy5.5® acid]	5 mg	678	701
174	Cyanine 5.5 monosuccinimidyl ester [equivalent to Cy5.5® NHS ester]	1 mg	678	701
283	Cyanine 5.5 monosuccinimidyl ester, potassium salt [same as Cy5.5® NHS ester]	1 mg	678	701
164	Cyanine 7 alkyne [equivalent to Cy7® alkyne]	1 mg	749	776

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Product Ordering Information For Near Infrared, Infrared and Classic Fluorescent Labeling Dyes

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
165	Cyanine 7 amine [equivalent to Cy7® amine]	1 mg	749	776
163	Cyanine 7 azide [equivalent to Cy7® azide]	1 mg	749	776
169	Cyanine 7 bisacid [equivalent to Cy7® bisacid]	5 mg	749	776
170	Cyanine 7 bissuccinimidyl ester [equivalent to Cy7® bisNHS ester]	1 mg	749	776
295	Cyanine 7 bissuccinimidyl ester, potassium salt [same as GE Cy7® bisNHS ester]	1 mg	749	776
166	Cyanine 7 hydrazide [equivalent to Cy7® hydrazide]	1 mg	749	776
162	Cyanine 7 maleimide [equivalent to Cy7® maleimide]	1 mg	749	776
160	Cyanine 7 monoacid [equivalent to Cy7® acid]	5 mg	749	776
161	Cyanine 7 monosuccinimidyl ester [equivalent to Cy7® NHS ester]	1 mg	749	776
290	Cyanine 7 monosuccinimidyl ester, potassium salt [same as GE Cy7® NHS ester]	1 mg	749	776
135	FITC-xtra	5 mg	492	515
136	FITC-xtra	25 mg	492	515
189	ICG acid	5 mg	780	800
188	ICG amine	1 mg	780	800
186	ICG Xtra-Osu	1 mg	780	800
181	ICG-ATT [3-ICG-acyl-1,3-thiazolidine-2-thione]	1 mg	780	800
182	ICG-OSu	1 mg	780	800
185	ICG-PEG12-OSu	1 mg	780	800
183	ICG-Sulfo-EG4-OSu	1 mg	780	800
184	ICG-Sulfo-EG8-OSu	1 mg	780	800
180	ICG-Sulfo-OSu	1 mg	780	800
1039	iFluor™ A7 SE	1 mg	758	784
470	Lissamine Rhodamine B Sulfonyl Chloride [Sulforhodamine B sulfonyl chloride] *CAS 62796-29-6*	100 mg	568	583
471	LRB Red™ SE	10 mg	568	583
715	OG488 maleimide [equivalent to Oregon Green® 488 maleimide]	5 mg	496	524
480	Sulforhodamine 101 sulfonyl chloride [Texas Red®] *CAS#: 82354-19-6*	10 mg	588	601
478	SunRed™ SE	1 mg	583	603
472	SunRed™ SE	5 mg	583	603
485	Texas Red® alkyne *Single Isomer*	5 mg	588	601
484	Texas Red® azide *Single Isomer*	5 mg	588	601
482	Texas Red® cadaverine *Single Isomer*	5 mg	582	602
481	Texas Red® hydrazide *Single Isomer*	5 mg	582	602
483	Texas Red® maleimide *Single Isomer*	5 mg	588	601
475	Texas Red-X, succinimidyl ester *Mixed isomers* *CAS 216972-99-5*	5 mg	588	601
474	Texas Red-X, succinimidyl ester *Single isomer* *CAS 199745-67-0*	5 mg	588	601

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Phycobiliproteins and Their Tandem Conjugates

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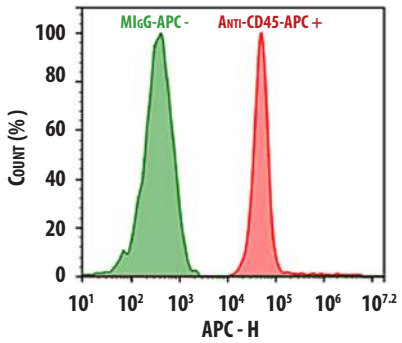

Figure 6.1 Flow cytometry analysis of HL-60 cells stained with 1 µg/ml Mouse IgG-APC Control (Green) or with 1 µg/ml Anti-Human CD45-APC (Red) prepared with Buccutite™ Rapid APC Antibody Labeling Kit (Cat#1311). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC channel.

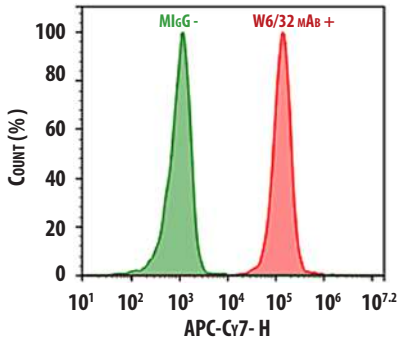

Figure 6.2 Flow cytometry analysis of HL-60 cells stained with 1 µg/ml Mouse IgG control (Green) or with 1ug/ml mouse Anti-Human HLA-ABC (W6/32 mAb) (Red) and then followed by Goat Anti-Mouse IgG-APC-Cy7 conjugate prepared with Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit (Cat#1321). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC-Cy7 channel.

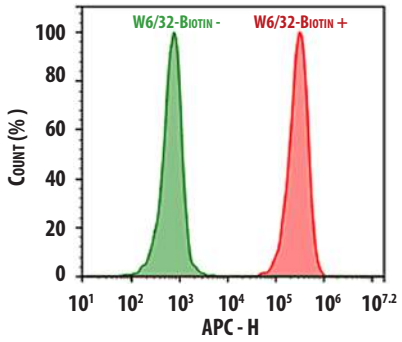

Figure 6.3 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1 µg/ml Anti-Human HLA-ABC-Biotin and then followed by APC-streptavidin conjugate (Cat#16902). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the APC channel.

Phycobiliporteins, derived from cyanobacteria and eukaryotic algae, are water-soluble complexes composed of a protein backbone and a covalently bound linear tetrapyrrole chromophore. Tetrapyrroles play a crucial role in absorbing light, and through FRET, they are able to transfer this energy to a pair of chlorophyll molecules. Phycobiliproteins are divided into two distinct classes: phycoerythrins, which exhibit a bright red fluorescence, and phycocyanins which fluoresce blue. Phycobiliproteins are relatively stable at room temperature and in a neutral pH. Howbeit, under acidic or basic conditions, purified phycobiliproteins will dissociate into their smaller subunits, resulting in a less intense coloration and fluorescence.

Phycobiliproteins such as B-phycoerythrin (B-PE), R-phycoerythrin (R-PE) and allophycocyanin (APC), are ultra-sensitive fluorescent dyes for biological detections. They are over 100 times more sensitive than conventional organic fluorophores, such as fluorescein. In practical applications such as flow cytometry and immunoassays, the sensitivity of phycobiliprotein-conjugated antibodies are usually much greater than that of the corresponding organic molecule-based conjugates. Phycobiliproteins are the brightest fluorescent tags with multiple sites for forming stable conjugation to many biological and synthetic materials.

Allophycocyanin and Its Tandem

Allophycocyanin (APC) is a 105 kDa phycobiliprotein isolated from blue-green algae. APC exhibits a bright red fluorescence, compliments of its 6 phycocyanobilin chromophores, with extremely high absorptivity and relatively high quantum efficiency. APC can be easily linked to proteins by conventional cross-linking techniques without impeding its spectral characteristics, and is suitable for applications such as flow cytometry.

Our APC-iFluor™ 700 Tandem is an excellent replacement for APC-Alexa Fluor® 700 Tandem since they have almost identical spectra. On some antibodies, our APC-iFluor™ 700 Tandem is much brighter compared to APC-Alexa Fluor® 700 Tandem with a higher stain index. Our APC-iFluor™ 750 Tandem is an excellent replacement for APC-Cy7® and APC-Alexa Fluor® 750 Tandem due to their similar spectral properties. Our APC-iFluor™ 750 Tandem is more photostable than APC-Cy7® Tandem.

R-Phycoerythrin and Its Tandem

R-Phycoerythrin (PE) is isolated from red algae and contains three types of subunits: α (~20,000 Daltons), β (~20,000 Daltons) and γ (~30,000 Daltons). PE is the most intensely

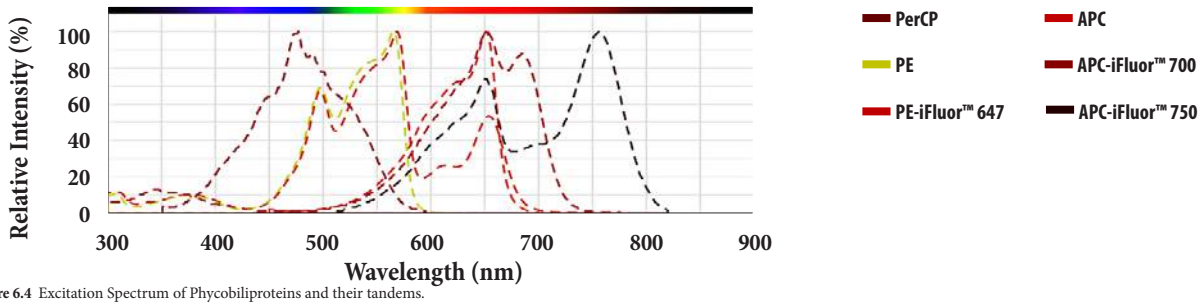

Figure 6.4 Excitation Spectrum of Phycobiliproteins and their tandems.

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fluorescent phycobiliproteins with quantum efficiencies probably in excess of 90%. Its orange fluorescence is readily visible by eye in any moderately concentrated solution. Each PE exists *in vitro* as a 240-kDa protein with 23 phycoerythrobilin chromophores per molecule. This makes PE the brightest fluorochrome for flow cytometry applications but its photobleaching properties make it unsuitable for fluorescence microscopy.

Our PE-iFluor™ 647 Tandem is an excellent replacement for PE-Cy5® and PE-Alexa Fluor® 647 Tandem since they have almost identical spectral properties. Our PE-iFluor™ 750 Tandem is an excellent replacement for PE-Cy7® and PE-Alexa Fluor® 750 Tandem due to their similar spectral properties. In general, our PE tandems have less residual PE fluorescence, and are more photostable (in some cases) compared to PE-Cy7® and PE-Alexa Fluor® 750 Tandems.

Peridinin-Chlorophyll-Protein Complex and Its Tandem

Peridinin-Chlorophyll-Protein (PerCP) is a component of the photosynthetic apparatus found in the dinoflagellate Glenodinium. PerCP is a protein complex with a molecular weight of approximately 35 kDa. Due to its photobleaching characteristics, PerCP conjugates are not recommended for use in flow cytometers with high-power lasers (>25 mW). Our PerCP-iFluor™ 700 Tandem is an excellent replacement for PerCP-Cy5.5® due to their similar spectra. PerCP-iFluor™ 700 Tandem is not subject to photobleaching like PerCP and can be used with stream-in-air flow cytometers. In addition, the PerCP- iFluor™ 700 tandem conjugate is not as susceptible to fixative or light instability compared to APC-Cy7® and PE-Cy7®.

Preactivated Phycobiliproteins and Its Tandems

To facilitate the conjugation of phycobiliproteins to proteins such as antibodies or streptavidin, AAT Bioquest offers pre-activated forms of phycobiliproteins, phycobiliproteins-iFluor dyes and phycobiliprotein-iFluor™ tandems. ReadiUse™ pre-activated phycobiliproteins have been pre-activated with our proprietary Buccutite™ crosslinking technology to provide a much higher conjugate yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our phycobiliproteins are conjugated to a protein via its amino groups which are readily abundant in proteins while SMCC chemistry targets thiol groups which are generated by the reduction of disulfides. Our Buccutite™ bioconjugation system is more robust and easier to use and enables faster and quantitative conjugation of biomolecules with higher efficiencies and yields

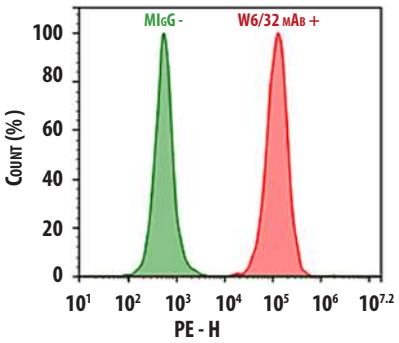

Figure 6.5 Flow cytometry analysis of HL-60 cells stained with 1 µg/ml Mouse IgG control (Green) or with 1 µg/ml mouse Anti-Human HLA-ABC (W6/32 mAb) (Red) and then followed by Goat Anti-Mouse IgG-PE conjugate prepared with Buccutite™ Rapid PE Antibody Labeling Kit (Cat#1310). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the PE channel.

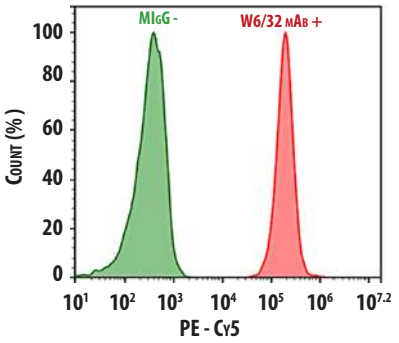

Figure 6.6 Flow cytometry analysis of HL-60 cells stained with 1µg/ml Mouse IgG control (Green) or with 1 µg/ml mouse Anti-Human HLA-ABC (W6/32 mAb) (Red) and then followed by Goat Anti-Mouse IgG-PE-Cy5 conjugate prepared with Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit (Cat#1326).

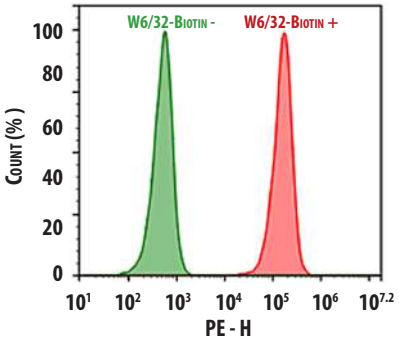

Figure 6.7 Flow cytometry analysis of HL-60 cells stained with (Red) or without (Green) 1 µg/ml Anti-Human HLA-ABC-Biotin and then followed by PE-streptavidin conjugate (Cat#16901). The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the PE channel.

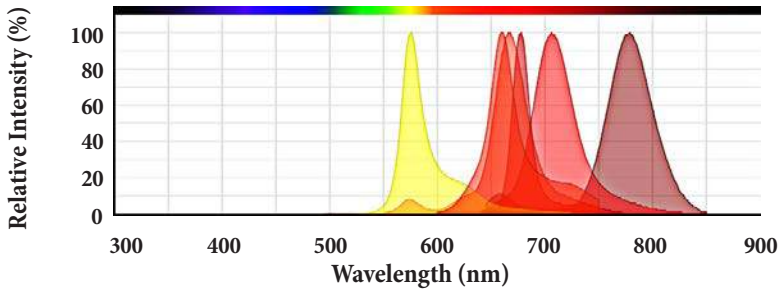

Figure 6.8 Emission Spectrum of Phycobiliproteins and their tandems.

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Product Ordering Information For Phycobiliproteins and Their Tandems

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
2552	CL-APC [Cross linked-AlloPhycocyanin]	1 mg	651	662
2553	C-PC [C-Phycocyanin] *CAS 11016-15-2*	1 mg	616	647
2554	APC [Allophycocyanin]	1 mg	651	662
2558	PE [R-Phycoerythrin] *CAS 11016-17-4*	1 mg	565	575
2559	PerCP [Peridinin-Chlorophyll-Protein Complex]	1 mg	482	677
2560	ReadiUse™ Preactivated PE	1 mg	565	575
2561	ReadiUse™ Preactivated APC	1 mg	651	713
2570	ReadiUse™ Preactivated APC-iFluor™ 700 Tandem	1 mg	651	713
2571	ReadiUse™ Preactivated APC-iFluor™ 750 Tandem	1 mg	651	779
2577	ReadiUse™ Preactivated PE-iFluor™ 647 Tandem	1 mg	565	674
2578	ReadiUse™ Preactivated PE-iFluor™ 750 Tandem	1 mg	565	779
2580	ReadiUse™ Preactivated PE-Cy5 Tandem	1 mg	565	670
2581	ReadiUse™ Preactivated PE-Cy5.5 Tandem	1 mg	565	700
2582	ReadiUse™ Preactivated PE-Cy7 Tandem	1 mg	565	780
2583	ReadiUse™ Preactivated PE-Texas Red Tandem	1 mg	565	600
2586	ReadiUse™ Preactivated APC-Cy5.5 Tandem	1 mg	651	700
2587	ReadiUse™ Preactivated APC-Cy7 Tandem	1 mg	651	780
2610	PE-Cy5 Tandem	1 mg	565	670
2613	PE-Cy5.5 Tandem	1 mg	565	700
2616	PE-Cy7 Tandem	1 mg	565	780
2619	PE-Texas Red Tandem	1 mg	565	600
2622	APC-Cy5.5 Tandem	1 mg	651	700
2625	APC-Cy7 Tandem	1 mg	651	780

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ReadiLink™ Protein Labeling Kits

Key Features of ReadiLink™ Kits

- **Comprehensive** - each kit includes all the essential components and optimized labeling protocols necessary for successful conjugation.
- **Simple** - only two steps required: a simple mixing step and spinning step.
- **Rapid** - minimal hands on time, less than 10 minutes.

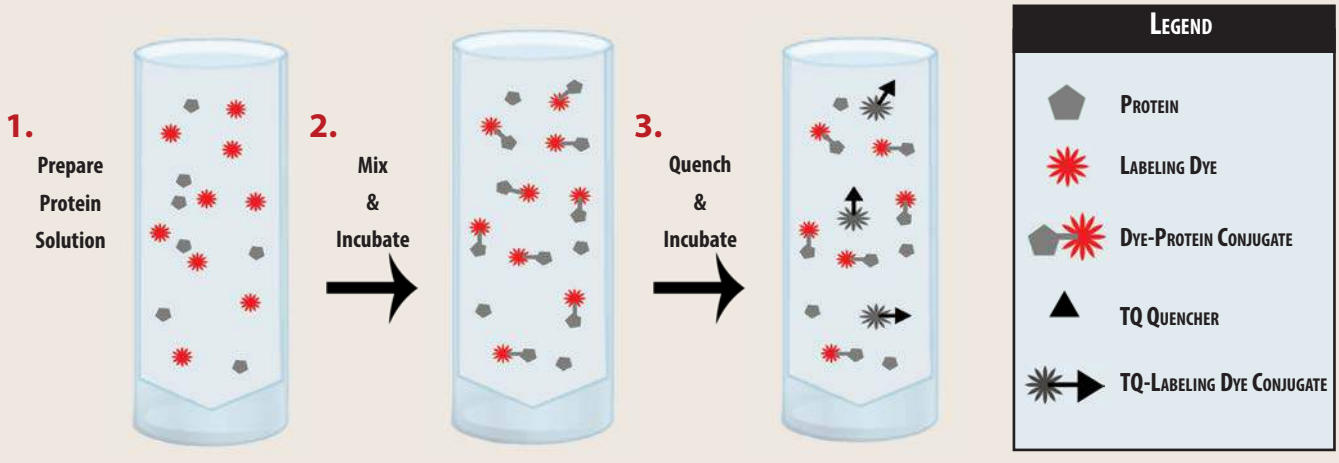
ReadiLink™ iFluor™ Protein Labeling Kits provide a convenient way to label proteins using one of the reactive forms of our iFluor™ labeling dyes and other small organic dyes. The dyes selectively react with the aliphatic amines of proteins forming a carboxamide bond, which is identical to and as stable as natural peptide bonds. The free and unconjugated dyes are mostly quenched with a non-fluorescent dye to eliminate any residual fluorescence. Dye-protein conjugates may be used for immunofluorescent staining, fluorescent in situ hybridization, flow cytometry and other biological applications.

AAT Bioquest offers a family of ReadiLink™ protein labeling kits spanning the entire visible spectrum range. Each kit comes with all the essential components necessary for performing the conjugation reaction. The kits provide a rapid method for preparing protein conjugates that can be used for fluorescence imaging and flow cytometry applications.

ReadiLink™ Kit Labeling Principle

1. ***Start*** the labeling reaction by mixing a labeling dye with a protein of choice in the Reaction Buffer (pH 7.5-8.5).
2. ***Incubation*** allows time for labeling reaction to occur, developing a mixture of dye-labeled protein conjugates and unreactive free dye.
3. ***Quench*** the reaction by mixing a non-fluorescent Tide Quencher™ (TQ) dye with the reaction solution. The TQ dye stops the reaction AND converts the unreactive free labeling dye to the non-fluorescent TQ-Labeling dye complex, which eliminates the background fluorescence interference of the free labeling dye.

ReadiLink™ Labeling Principles



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ReadiLink™ iFluor™ Labeling Kits

AAT Bioquest's ReadiLink™ iFluor™ protein labeling kits provide a convenient method to label proteins (>10 kDa) such as monoclonal or polyclonal antibodies with our iFluor™ labeling dyes. iFluor™ labeling dyes are bright and photostable over a broad pH range with little pH sensitivity and minimal quenching on proteins. They can be well excited by the major laser lines of fluorescence instruments (e.g., 350, 405, 488, 555 and 633 nm).

ReadiLink™ iFluor™ labeling kits do not require any intermediate purification and can be easily completed in two simple mixing steps. iFluor™ dyes used in ReadiLink™ kits are conjugated to a reactive moiety (succinimidyl ester) and demonstrate great reactivity and selective targeting of protein amino groups. Each ReadiLink™ kit has been optimized to include a robust bioconjugation system and all the essential components necessary for two labeling of 50 µg antibodies each. The iFluor™ dye-labeled conjugates may be used for immunofluorescent staining, fluorescence in situ hybridization, flow cytometry and other biological applications.

ReadiLink™ mFluor™ Labeling Kits

AAT Bioquest's ReadiLink™ mFluor™ protein labeling kits provide a convenient method to label proteins (>10 kDa) such as monoclonal or polyclonal antibodies with the mFluor™ labeling dyes. Our ReadiLink™ mFluor™ protein labeling kits are developed and optimized for flow cytometry-focused applications. The succinimidyl esters (SE) of mFluor™ dyes used in these kits exhibit great reactivity and selective targeting of the aliphatic amines of proteins to form a carboxamide bond, which is identical to and as stable as natural peptide bonds.

mFluor™ labeling dyes exhibit large Stokes Shifts, and can be well excited by the common laser lines of flow cytometers (e.g., 405 nm, 488 nm and 633 nm). Each ReadiLink™ kit includes a robust bioconjugation system and all the essential components necessary to perform five separate labeling reactions and purifications. Each of the two mFluor™ labeling dyes have been optimized for labeling ~50 µg of antibodies. The resulting mFluor™ dye-labeled conjugates may be used for immunofluorescent staining, fluorescence in situ hybridization, flow cytometry and other biological applications.

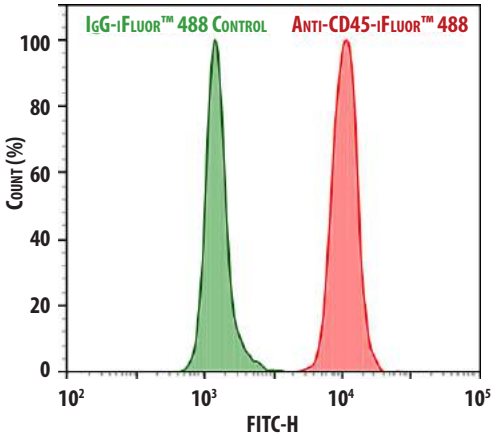

Figure 7.1 Expression of CD45 in differentiated HL-60 cells were quantified using labeled anti-CD45 antibody by ReadiLink™ Rapid iFluor™ 488 Antibody Labeling Kit (Cat# 1255). HL-60 cells were treated with 1.25% DMSO for 4 days to differentiate. The live cells were incubated with 1 µg/ml anti-CD45-iFluor™ 488 or IgG-iFluor™ 488 control and analyzed by NovoCyte.

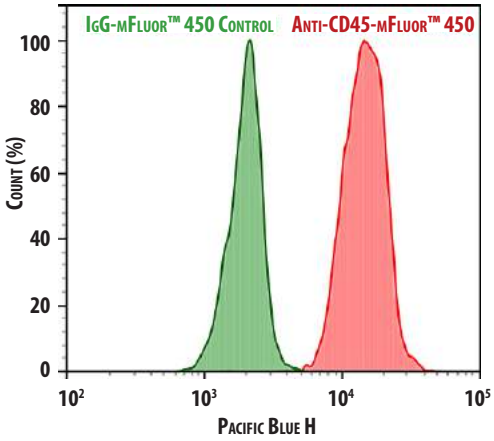

Figure 7.2 Expression of CD45 in differentiated HL-60 cells were quantified using labeled anti-CD45 antibody by ReadiLink™ Rapid mFluor™ Violet 450 Antibody Labeling Kit (Cat# 1100). HL-60 cells were with 1.25% DMSO for 4 days to differentiate. The live cells were incubated with 1 µg/ml anti-CD45-mFluor™ 450 or IgG-mFluor™ 450 control and analyzed by NovoCyte.

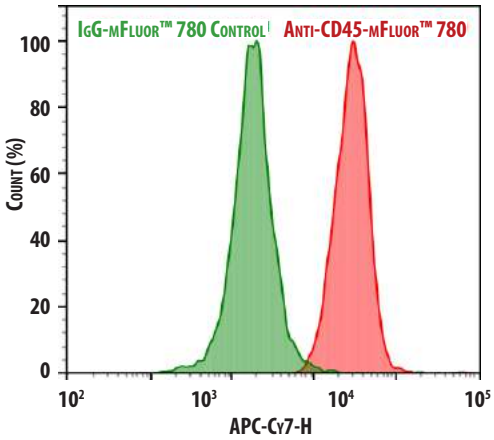

Figure 7.3 Expression of CD45 in differentiated HL-60 cells were quantified using labeled anti-CD45 antibody by ReadiLink™ Rapid mFluor™ Red 780 Antibody Labeling Kit (Cat# 1131). HL-60 cells were treated with 1.25% DMSO for 4 days to differentiate. The live cells were incubated with 1 µg/ml anti-CD45-mFluor™ 780 or IgG-mFluor™ 780 control and analyzed by NovoCyte.

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Product Ordering Information For ReadiLink™ Labeling Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
1299	ReadiLink™ FITC Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	492	516
1220	ReadiLink™ Rapid iFluor™ 350 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	345	442
1255	ReadiLink™ Rapid iFluor™ 488 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	491	514
1227	ReadiLink™ Rapid iFluor™ 555 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	559	569
1230	ReadiLink™ Rapid iFluor™ 594 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	592	614
1260	ReadiLink™ Rapid iFluor™ 633 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	638	655
1235	ReadiLink™ Rapid iFluor™ 647 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	654	674
1240	ReadiLink™ Rapid iFluor™ 680 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	682	701
1245	ReadiLink™ Rapid iFluor™ 700 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	693	713
1250	ReadiLink™ Rapid iFluor™ 750 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	753	779
1265	ReadiLink™ Rapid iFluor™ 790 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	782	811
1120	ReadiLink™ Rapid mFluor™ Blue 570 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	553	570
1123	ReadiLink™ Rapid mFluor™ Green 620 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	522	617
1130	ReadiLink™ Rapid mFluor™ Red 700 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	657	700
1131	ReadiLink™ Rapid mFluor™ Red 780 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	629	780
1105	ReadiLink™ Rapid mFluor™ Violet 420 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	398	411
1100	ReadiLink™ Rapid mFluor™ Violet 450 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	403	454
1110	ReadiLink™ Rapid mFluor™ Violet 510 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	414	508
1114	ReadiLink™ Rapid mFluor™ Violet 540 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	405	537
1126	ReadiLink™ Rapid mFluor™ Yellow 630 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	561	630
1300	ReadiLink™ Rapid trFluor™ Eu Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	346	617
1305	ReadiLink™ Rapid trFluor™ Tb Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	2 Labelings	330	544

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Buccutite™ Crosslinking Technology

Buccutite™ Conjugation Vs. SMCC Conjugation

Total Operation time:
- **Buccutite™ Chemistry:** 2-4 hours
- **SMCC Chemistry:** 4-8 hours

Minimum Sample Concentration:
- **Buccutite™ Chemistry:** ≥ 0.5 mg/ml
- **SMCC Chemistry:** ≥ 5 mg/ml

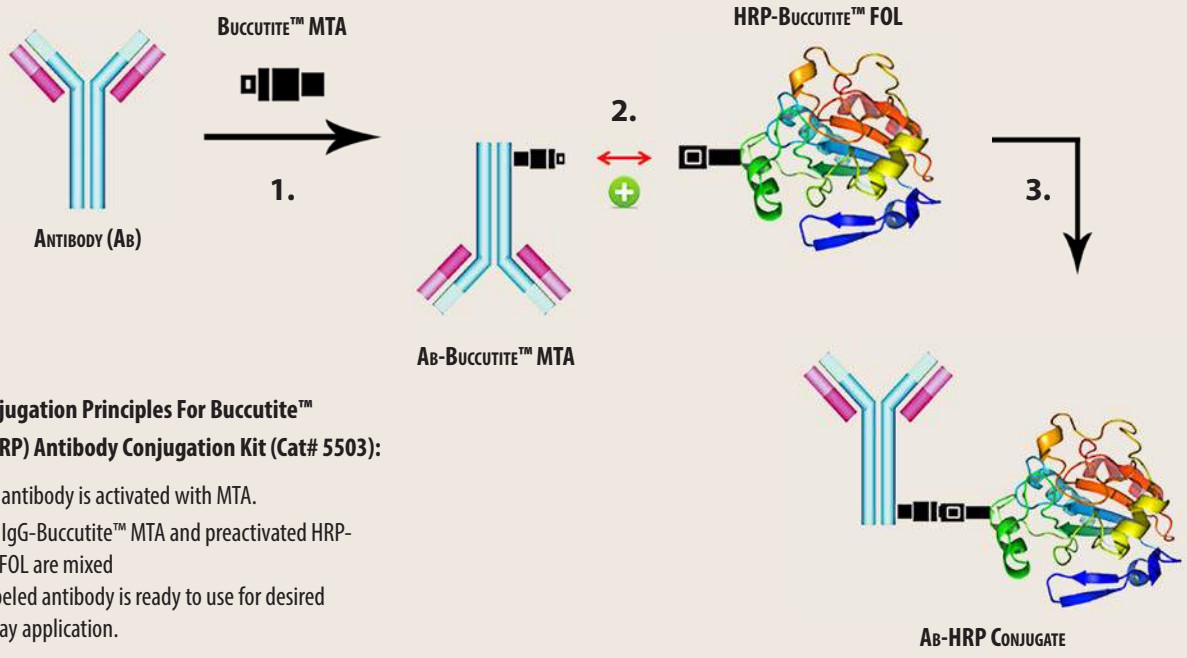
Optimal Conjugation pH:
- **Buccutite™ Chemistry:** 5-10
- **SMCC Chemistry:** 5-6

Conjugation Yield:
- **Buccutite™ Chemistry:** High
- **SMCC Chemistry:** Moderate

The modification and labeling of a protein with a tag macromolecule is an important task in biological research. There are a few methods available for crosslinking two macromolecules. For example, the two macromolecules can be directly linked. This method is not preferred, however, because it has extremely low yields and is very difficult to purify. Instead, a common crosslinking technique uses a small crosslinker such as SMCC to link macromolecules. One end of the SMCC consists of an NHS ester which reacts with amines (-NH_2) found in the amino acid lysine and N-terminus. The other end consists of maleimide moieties which reacts with the thiol groups (-SH) found in the amino acid cysteine. Although SMCC chemistry has been widely used in numerous bioconjugations, SMCC-modified proteins are extremely unstable during storage since proteins often contain both amine and thiol groups leading to significant amounts of homo-crosslinking. In addition, it is quite difficult and tedious to quantify the number of thiol groups on a protein.

AAT Bioquest has recently developed an effective crosslinking method to selectively link two macromolecules with a high conjugation yield. This new crosslinking method uses two unique crosslinkers that readily react upon mixing. More specifically, the pair of crosslinkers used includes our proprietary Buccutite™ FOL, SE and Buccutite™ MTA, SE. A protein, such as an antibody, is first labeled with the Buccutite™ FOL, SE and the tag macromolecule is labeled with the Buccutite™ MTA, SE. The two resulting Buccutite™-modified conjugates can be readily purified by a desalting column or dialysis. Mixing the Buccutite™ FOL-labeled protein and the Buccutite™ MTA-tag macromolecule under extremely mild conditions results in the desired protein-tag macromolecule conjugate. This process is illustrated in the diagram below.

Buccutite™ Crosslinking Principle



Buccutite Conjugation Principles For Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit (Cat# 5503):

1. Desired antibody is activated with MTA.
2. Actived IgG-Buccutite™ MTA and preactivated HRP-Buccutite™ FOL are mixed
3. HRP-labeled antibody is ready to use for desired immunoassay application.

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The Buccutite™ bioconjugation system is run at neutral pH, and completed within 1 hour. The conjugation can be run at low concentrations, thus eliminating the pretreatment step for some diluted proteins. This crosslinking reaction occurs under extremely mild neutral conditions without any catalyst required compared to some existing crosslinking chemistry (such as Solulink™), and it is much more robust and efficient.

Key Features of Buccutite™ Crosslinking Technologies

- Linkers are highly stable. The Buccutite™ linker-activated macromolecules are very stable, can be stored at 4 °C for more than 24 months.
- Conjugation conditions are extremely mild and robust.
- Buccutite™ conjugations can be run in a broad range of pH, concentration and temperature. No catalyst is required.
- High yield: Buccutite™ conjugation is fast and efficient. Conjugation is completed within 1 hour. Buccutite™ conjugation gives much higher yield than other existing methods under the same conditions. The conjugation can be run at extremely low concentration.

AAT Bioquest's Custom Bioconjugation Services

Together We Shine℠

AAT Bioquest offers same-day custom conjugation of proteins or antibodies with a wide array of labels, such as biotin, HRP and over 20 fluorophores. Our services are designed to produce high-quality results with a fast turnaround. We take pride in supplying scientists with flexible tools that help expand their research needs.

Features of AAT Bioquest's custom bioconjugation services include:

- Guaranteed quality with 95% purity rating
- Affordable price tiers that work with your budget
- Scalable service with minimum order of 50 µg
- Same day order fulfilment means your conjugate ships the day you order it

NOTE: APC and PE custom conjugation services require 3 working days

You may supply your own protein or antibody or chose from 3000+ monoclonal and polyclonal antibodies in our catalog. Contact us for a quote today, and let us put our expertise to work for you!

Custom Bioconjugation Services

How Does it Work?

Custom bioconjugates allow for the study of specific analytes in a complex biological systems Proteins, such as antibodies, can be conjugated to reporter enzymes or with a variety of fluorescent labels for use in applications such as Western Blot and flow cytometry.

Picking the Right Label

Picking the correct label to use for your experiment can be difficult, especially when there are so many choices. Here are some considerations to keep in mind:

Single/Multiplex Format:

- **Explanation:** If you are running a multiplex experiment, be aware of spectral overlaps. Too much overlap will lead to signal bleed-through between fluorophores.
- **Our Suggestion:** Use our Spectrum Viewer web application to compare fluorophore excitation/emission and minimize overlaps.

Instrumentation:

- **Explanation:** Make sure that your instrument can properly detect the chosen fluorophore. Is it colorimetric or fluorimetric? Does it have the proper excitation sources? Does it have the correct filters?
- **Our Suggestion:** Check our fluorophore product page for specific spectral properties to see if it is compatible with your instrumentation.

Experimental Conditions:

- **Explanation:** Does your experiment require special conditions such as temperature or pH? Many fluorophores are sensitive to experimental conditions, which will impact their brightness and susceptibility to photo-bleaching.
- **Our Suggestion:** Check our fluorophore product page for specific restrictions on usage.

Available Labels

AAT Bioquest offers a comprehensive list of fluorophores, reporter enzymes, tandem dyes and biotin for custom conjugations.

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Product Ordering Information For Buccutite™ Labeling Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
5503	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 100 µg Protein*	1 kit	N/A	N/A
1313	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	651	662
1311	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	651	662
1322	Buccutite™ Rapid APC-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	651	670
1350	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	651	700
1320	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	651	700
1351	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	651	780
1321	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	651	780
1347	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	651	713
1319	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	651	713
1312	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	565	575
1310	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	565	575
1340	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	565	670
1341	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	565	700
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	565	700
1342	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	565	780
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	565	780
1353	Buccutite™ Rapid PerCP Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	482	677
1325	Buccutite™ Rapid PerCP Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	482	677
1343	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings	565	600
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings	565	600
1315	Buccutite™ Rapid Protein Crosslinking Kit *Microscale Optimized for Crosslinking 100 µg Antibody Per Reaction*	2 Crosslinkings	N/A	N/A

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Appendix

Appendix 1. Spectral Properties of iFluor™ Fluorescent Labeling Dyes

Labeling Dye	Extinction Coefficient[1] ($cm^{-1}M^{-1}$)	Abs (nm)	Em (nm)	FQY[2]	CF at 280 nm[4]
iFluor™ 350	20,000	345	442	0.95	0.187
iFluor™ 405	29,000	401	420	0.91	0.697
iFluor™ 488	75,000	491	514	0.9	0.139
iFluor™ 514	80,000	518	542	0.95	0.182
iFluor™ 532	81,000	542	558	0.9	0.192
iFluor™ 555	150,000	555	565	0.105	0.073
iFluor™ 594	110,000	594	614	0.155	0.07
iFluor™ 610	90,000	605	627	0.85	0.441
iFluor™ 633	250,000	638	655	0.24	0.045
iFluor™ 647	250,000	649	665	0.25	0.03
iFluor™ 680	220,000	676	695	0.18	0.101
iFluor™ 700	220,000	685	710	0.2	0.036
iFluor™ 750	275,000	749	775	0.13	0.036
iFluor™ 790	250,000	782	811	0.09	0.091
iFluor™ A7	275,000	758	784	0.15	0.022

Appendix 2. Spectral Properties of mFluor™ Fluorescent Labeling Dyes

Labeling Dye	Extinction Coefficient[1] ($cm^{-1}M^{-1}$)	Abs (nm)	Em (nm)	CF at 280 nm[4]
mFluor™ Blue 570	170,000	553	570	0.191
mFluor™ Green 620	60,000	522	617	0.554
mFluor™ Red 700	295,000	657	700	0.096
mFluor™ Red 780	90,000	629	780	0.081
mFluor™ Violet 450	35,000	403	454	0.238
mFluor™ Violet 510	25,000	414	508	0.781
mFluor™ Violet 540	21,000	399	540	0.661
mFluor™ Violet 630	110,000	561	630	0.391

Note. 1. Extinction Coefficient at their maximum absorption wavelength; 2. FQY = fluorescence quantum yield in aqueous buffer (pH 7.2); 3. CF at 280 nm is the correction factor used for eliminating the dye contribution to the absorbance at 280 nm (for peptide and protein labeling); 4. Fluorescence intensity is significantly increased upon coupling to proteins.

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Website: http://www.cosmobio.co.jp

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Website: http://www.nacalai.com

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Website: http://www.kimnfriends.co.kr

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Email: info@biosite.se
Website: http://www.biosite.se

Netherlands:
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Email: info@itk.nl
Website: http://www.itk.nl

Norway:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

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Website: http://www.lubio.ch

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Website: http://www.biomol.de

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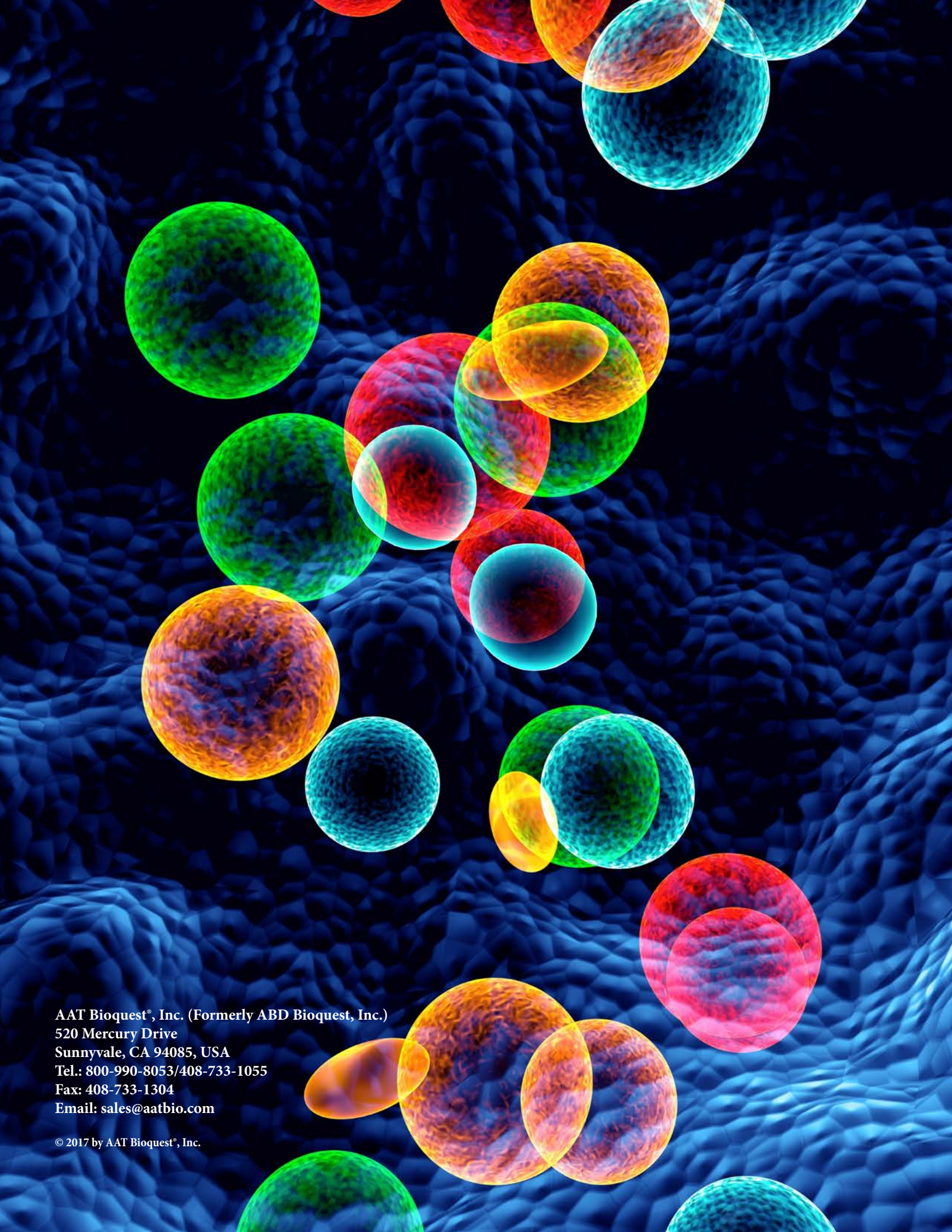

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