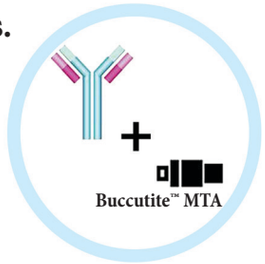
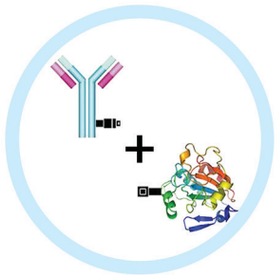
Buccutite™ Crosslinking Principles

Conjugate Proteins In 2 Simple Mixing Steps.

*NO catalyst required



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.

*Buccutite™ Conjugation using Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit (Catalog: 5503)

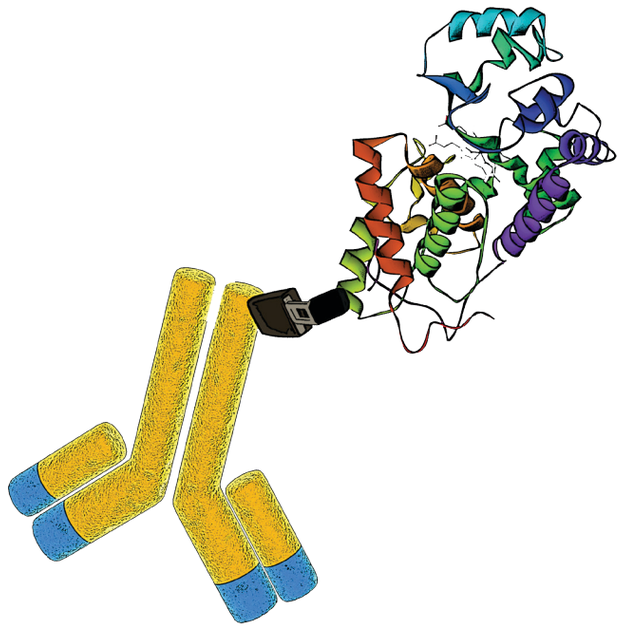
Product Ordering Information For Buccutite™ Labelling Kits

Cat #	Product Name	Abs/Em (nm)
5503	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 100 µg Protein*	N/A
1313	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 2w5 µg Antibody Per Reaction*	651/662
1311	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651/662
1322	Buccutite™ Rapid APC-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651/670
1350	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	651/700
1320	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651/700
1351	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	651/780
1321	Buccutite™ Rapid APC-Cy7 Tandem Antwibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651/780
1347	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	651/713
1319	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651/713
1312	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	565/575
1310	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565/575
1340	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	565/670
1341	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	565/700
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565/700
1342	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	565/700
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565/780
1353	Buccutite™ Rapid PerCP Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	482/677
1325	Buccutite™ Rapid PerCP Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	482/677
1343	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	565/600
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565/600
1315	Buccutite™ Rapid Protein Crosslinking Kit *Microscale Optimized for Crosslinking 100 µg Antibody Per Reaction*	N/A

For more information contact us at **1-(800)-990-8053** or visit our website at www.aatbio.com

Buccutite™ Bioconjugation Technology

Rapid Protein Crosslinking Method for Labeling and Modifying Antibodies



AAT Bioquest®

Crosslinking Methods

Crosslinking is a technique that chemically joins two or more proteins together, such as an antibody and an enzyme, by a covalent bond. Crosslinking reagents consist of reactive moieties that modify and attach to specific functional groups on proteins. A common approach for conjugating two biomolecules utilizes a small, heterobifunctional crosslinker called succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC). SMCC crosslinkers contain amine-reactive NHS ester moieties and thiol-reactive maleimide moieties to crosslink proteins. However, a drawback to this approach is the many experimental conditions that must be monitored to perform a successful conjugation. Difficulties associated with SMCC conjugations are maintenance of a narrow pH range, self-polymerization, and hydrolytic degradation of reactive moieties. To address these concerns, AAT Bioquest has developed an optimized and robust Buccutite™ crosslinking technique. Buccutite™ crosslinking technology provides a simplistic and efficient approach for conjugating proteins. It can be completed in half the time and with less stringent parameters yielding highly stable and easy to handle conjugated proteins.

Advantages

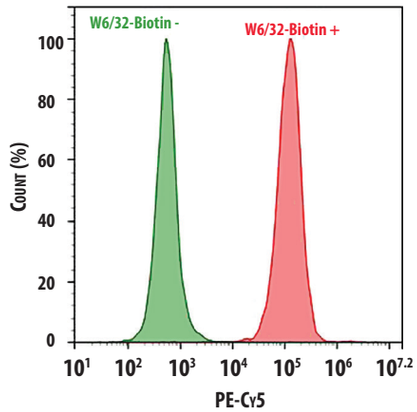
Fast
Buccutite™ conjugation can be completed in 1-2 hours under extremely mild conjugation condutions. No purification step required with 25µg kits.

Robust
Buccutite™ conjugation can be run in a broad pH range of 5-9 and temperature.

Highly Stable
Buccutite™ linker-activated macromolecules are very stable, can be stored at 4 °C for more than 24 months.

High Yield
Buccutite™ conjugation gives much higher yield than other existing methods under the same conditions. Eliminating homo-crosslinking of proteins.

Results



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

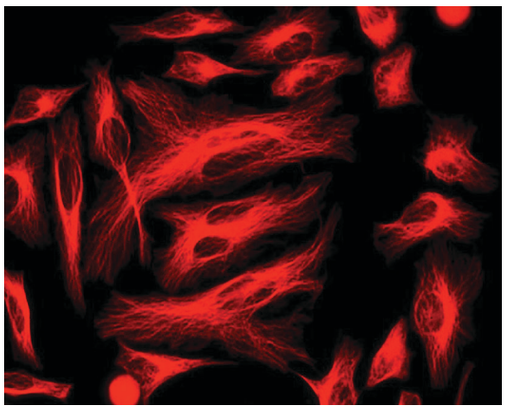
Buccutite™ vs SMCC

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

Minimum Sample Concentration:
- **Buccutite™ Chemistry:** ≥ 0.5 mg/mL
- **SMCC Chemistry:** 0.5 - 5.0 mg/mL

Optimal Conjugation pH:
- **Buccutite™ Chemistry:** 5 - 9
- **SMCC Chemistry:** 7.0 - 7.5

Conjugation Yield:
- **Buccutite™ Chemistry:** 50-60 %
- **SMCC Chemistry:** ≤ 30 %



Tubulins were imaged with RPE- goat-anti-mouse IgG conjugate in HeLa cells. Tubulins were stained using mouse anti-α-tubulin antibody, and visualized with red fluorescent RPE-goat-anti-mouse IgG conjugate prepared by Buccutite™ crosslinking technology as described above.

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!