Buccutite™

Crosslinking

Technology

Robust
Accurate
Proficient
Intuitive
Dependable

AAT Bioquest®

Stratech

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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Introduction

Buccutite™ crosslinking technology offers a truly unique and trouble-free method for labeling primary antibodies with another macromolecule, such as an enzyme or phycobiliprotein. By directly labeling a primary antibody with a fluorophore or enzyme tag it streamlines the immunostaining procedure by eliminating the need for secondary conjugates and the additional incubation and wash steps that follow. Furthermore, directly labeled conjugates exhibit less nonspecific binding, lower background interference, and enables the use of multiple primary antibodies from the same species for multicolor detection.

Compared to the conventional SMCC technique for protein-protein conjugation, which is a moderately difficult and low-yielding process, Buccutite™ conjugation is significantly more robust and less demanding. Conjugation takes 2 hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. To conjugate two proteins, Buccutite™ labeling kits use a pair of proprietary crosslinkers – Buccutite™ MTA and Buccutite™ FOL. Each crosslinker is independently labeled onto a primary antibody or protein of interest via its amine-reactive moiety. When mixed together, proteins will bind with a high degree of affinity and specificity to their Buccutite™ MTA/FOL counterpart. The covalent bond formed between the Buccutite™ linkers is extremely stable resulting in conjugates that can tolerate the rigorous washing process in a typical immunoassay (e.g. ELISA, IHC and Western blot) or flow cytometric application.

Buccutite™ labeling kits provide all the essential components needed to label 25 µg to 1 mg of antibody with a range of detectable tags including horseradish peroxidase (HRP), poly-HRP, alkaline phosphatase (AP), PE, APC, PerCP and tandem dyes.

Buccutite™ versus SMCC Conjugation at-a-Glance

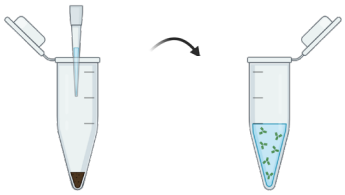
	Buccutite™	SMCC
Minimum antibody concentration	100 µg/mL	0.5 to 5 mg/mL
Labeling Chemistry	Uses two proprietary linkers, Buccutite™ MTA and Buccutite™ FOL, to facilitate protein-protein conjugation.	Uses a heterobifunctional crosslinker to facilitate protein-protein conjugation. Labeling protocol is tedious and requires existing knowledge of bioconjugation chemistry.
Available labels	PE, APC, Tandem Dyes, HRP, Poly-HRP and AP. Cat No 1315 can be used to conjugate any two proteins.	User is responsible for supplying proteins, method suitable for any protein-protein conjugation
Type of bond between label and antibody	Covalent	Covalent
Compatible with BSA or other stabilizers?	No	No
Requires purification?	No	Yes
Time required for conjugation	2 hrs	4 hrs or more
Hands-on-time	~15 minutes	2 hrs
Conjugate yield	100 %	~30%
Batch-to-batch variation	Minimal	High
Applications	FC, IF, IHC, WB, ELISA	FC, IF, IHC, WB, ELISA

Simple 5 Step Process

01

Add Buccutite™ Reaction Buffer to your antibody working solution.

Hands-on-time = ~1 minute



02

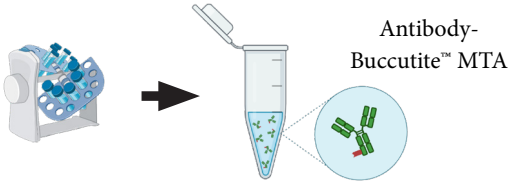
Reconstitute Buccutite™ MTA linker in DMSO and add to antibody working solution.

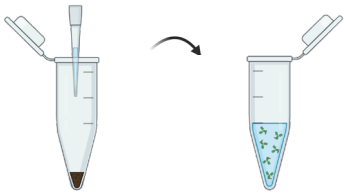
Hands-on-time = ~5 minute

Rotate antibody-Buccutite™ MTA reaction mixture for 30 to 60 minutes at room temperature.

Hands-on-time = ~1 minute

03





04

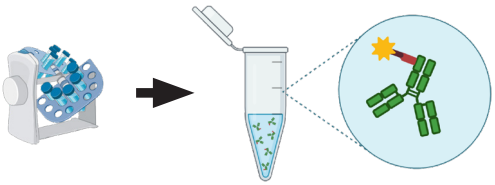
Reconstitute Buccutite™ FOL-Activated HRP, PE, APC or tandem in ddH_2O and mix with antibody-Buccutite™ MTA solution

Hands-on-time = ~5 minute

Rotate reaction mixture for 60 minutes at room temperature.

Hands-on-time = ~1 minute

05



Ready to use!

Buccutite™ HRP and Poly HRP Antibody Labeling Kits

Key Features

- Efficient method for covalently labeling 25 µg to 1 mg of antibody with HRP or poly-HRP
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for ELISA, IHC, blotting, Power Styramide Signal Amplification and TSA Imaging

The Buccutite™ Peroxidase (HRP) Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with the detection enzyme horseradish peroxidase (HRP). To streamline the process, HRP provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated HRP component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. For difficult applications that require an additional level of sensitivity, we offer Buccutite™ Poly-HRP Antibody Labeling Kits. These kits enable the direct labeling of antibodies to polymers of HRP, resulting in conjugates that deliver the highest sensitivity and signal-to-noise ratios. Poly-HRP conjugates are ideal for applications where the target of interest is in low-abundance or when sample volume is limited.

Each Buccutite™ Peroxidase (HRP) Antibody Labeling Kit and Buccutite™ Poly-HRP Antibody Labeling Kit provides sufficient reagents to perform either 1, 2 or 5 separate labelings of 25 µg, 50 µg, 100 µg or 1 mg of antibody each, including:

- Buccutite™ FOL-Activated HRP or Buccutite™ FOL-Activated Poly-HRP
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

Horseradish Peroxidase

Horseradish peroxidase is a 44 kDa metalloenzyme isolated from the roots of horseradish. It is used extensively as an enzyme reporter system in biochemistry applications, such as ELISA, for its high turnover rate, ability to amplify week signals and enhance the detectability of low abundance targets.

Alone, HRP or conjugates thereof, have little value as they lack the capacity to generate a measurable signal. To facilitate target visualization, HRP conjugates require compounds known as substrates. In the presence of hydrogen peroxide, HRP catalyzes the oxidation of these substrates to produce a quantifiable signal. HRP substrates are divided according to the type of signal it produces, these include, colorimetric, fluorimetric or chemiluminescent substrates.

Table 1. Properties of colorimetric, fluorescent and chemiluminescent HRP and poly-HRP substrates.

HRP Substrate	Abs (nm)	Em (nm)	Notes
ABTS	420 nm	N/A	• Readily oxidized • Slow color development
TMB	450 nm (stop reaction) 650 nm (kinetic assay)	N/A	• Readily oxidized • Low water solubility
Amplite™ Blue	324 nm	409 nm	• Sensitive, fast signal development • Resistant to photobleaching
Amplite™ ADHP [10-Acetyl-3,7-dihydroxyphenoxazine]	570 nm	583 nm	• Resistant to oxidation • Stable, sensitive, fast signal development
Amplite™ Red	570 nm	583 nm	• Resistant to oxidation • Sensitive fast signal development
Amplite™ IR	646 nm	667 nm	• Resistant to oxidation • Sensitive, fast signal development • pH-independent fluorescence (pH 4-10)
Luminol [3-Aminophthalhydrazide] *CAS 521-31-3*	N/A	410 nm	• Sensitive, fast signal development

* Absorption (Abs) and fluorescence emission maxima (Em), N/A = not applicable.

Concentration Curve

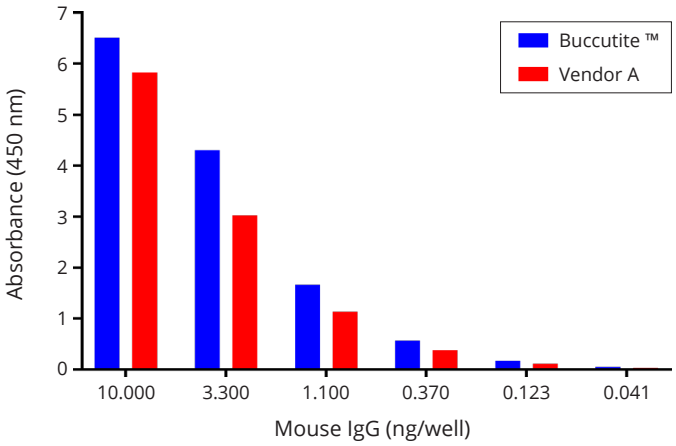


Figure 1. Detection of mouse IgG using goat anti-mouse HRP conjugates. Goat-anti mouse IgG antibody was labeled using either the Buccutite™ HRP Antibody Conjugation Kit (Cat No. 5503) or an HRP Antibody Labeling kit purchased from Vendor A. A 3-fold serial dilution of mouse IgG antibody was coated on a white wall/clear bottom 96-well plate. 100 µL goat anti-mouse IgG HRP conjugate (100 ng/mL) was tested using the standard ELISA method. 100 µL of ReadiUse™ TMB substrate solution (Cat No. 11000) was added to the wells and allowed to develop for 5 minutes. The reaction was stopped by the addition of 100 µL of 1.0 N HCl and read at 405 nm.

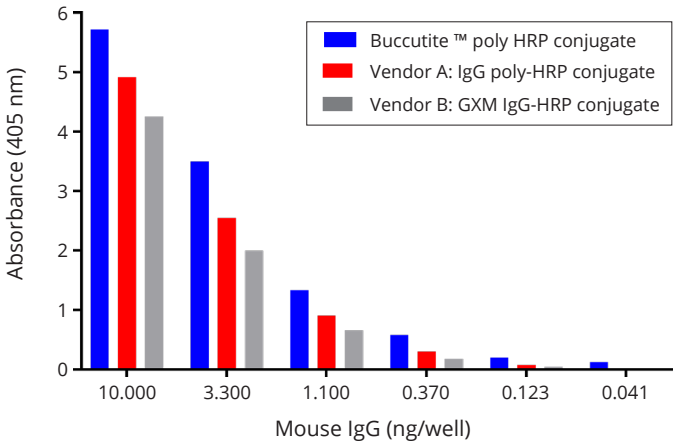


Figure 2. Detection of mouse IgG using goat anti-mouse IgG labeled with HRP or poly-HRP. Poly-HRP conjugates were prepared using Buccutite™ Poly-HRP Antibody Conjugation Kit (Cat No. 5519) or a poly-HRP Antibody Labeling Kit purchased from Vendor A, and a goat anti-mouse IgG HRP conjugate was purchased from Vendor B. A 3-fold dilution of mouse IgG antibody was coated on a white wall/clear bottom 96-well plate. 100 µL of each goat anti-mouse IgG polyHRP conjugate (100 ng/mL) and 100 µL goat anti-mouse IgG HRP was tested using the standard ELISA method. 100 µL of ReadiUse™ TMB substrate solution (Cat No. 11000) was added to the wells and allowed to develop for 5 minutes. The reaction was stopped by the addition of 100 µL of 1.0 N HCl and read at 405 nm.

Ordering Information

Cat No.	Product	Quantity
11000	Amplite™ ADHP [10-Acetyl-3,7-dihydroxyphenoxazine] *CAS#: 119171-73-2*	25 mg
11005	Amplite™ Blue	25 mg
11009	Amplite™ IR	1 mg
11011	Amplite™ Red	1000 Assays
5505	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 25 ug Protein*	2 x 25 µg
5503	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*	2 x 100 µg
5504	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	1 x 1 mg
5506	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	5 x 1 mg
5518	Buccutite™ Poly-HRP Antibody Conjugation Kit *Optimized for Labeling 50 µg Protein*	1 x 50 µg
5519	Buccutite™ Poly-HRP Antibody Conjugation Kit *Optimized for Labeling 50 µg Protein*	2 x 50 µg
11050	Luminol [3-Aminophthalhydrazide] *CAS 521-31-3*	1 g
11013	ReadiUse™ ABTS Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	100 mL
11001	ReadiUse™ ABTS Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	1 L
11012	ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	100 mL
11003	ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	1 L
11010	Signal Guard™ HRP conjugate stabilizer	50 mL
11020	Signal Guard™ HRP reaction stopping solution	0.5 mL

Buccutite™ AP Antibody Labeling Kits

Key Features

- Efficient method for covalently labeling 25 µg to 1 mg of antibody with AP
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for ELISA, IHC, and blotting

The Buccutite™ AP (Alkaline Phosphatase) Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with the detection enzyme alkaline phosphatase (AP). To streamline the process, AP provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated AP component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody.

Each Buccutite™ AP (Alkaline Phosphatase) Antibody Labeling Kit provides sufficient reagents to perform 2 separate labelings of 25 µg, 100 µg or 1 mg of antibody each, including:

- Buccutite™ FOL-Activated AP
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

Alkaline Phosphatase

Alkaline phosphatase is a 86 kDa homodimeric enzyme that plays a key role in the dephosphorylation of organic compounds. It contains two zinc atoms per monomer, which are crucial to its catalytic function, and is optimally active at alkaline pH levels. AP has become a highly sensitive reporter enzyme in immunoassays such as ELISA, as well as, in immunohistochemical (IHC), Northern, Southern and Western blot applications. For these applications, AP is usually conjugated to a primary or secondary antibody and its activity is detected with a signal generating colorimetric, fluorimetric or chemiluminescent substrate.

Table 1. Properties of colorimetric, fluorescent and chemiluminescent AP substrates.

AP Substrate	Abs*	Ex*	Em*	Notes
FDP [Fluorescein diphosphate, tetraammonium salt] *CAS 217305-49-2*	487	498	517	• Most sensitive fluorogenic phosphatase substrate • Thermally unstable • Suitable for chromogenic detection
MUP [4-Methylumbelliferyl phosphate, free acid] *CAS 3368-04-5*	N/A	360	448	• Max signal development at pH > 10 • Not well-suited for living cell or continuous assays
MUP, disodium salt [4-Methylumbelliferyl phosphate, disodium salt] *CAS 22919-26-2*	N/A	360	448	• Max signal development at pH > 10 • Not well-suited for living cell or continuous assays
DiFMUP	N/A	354	450	• Lower pKa than MUP • Suitable for assaying acidic phosphatases at low pH
pNPP [4-Nitrophenyl phosphate, disodium salt] *CAS 4264-83-9*	405	N/A	N/A	• Chromogenic substrate • Suitable for both acid and alkaline phosphatase
PhosLite™ Green	N/A	345	520	• Water-soluble • Fast signal development • Highly photostable
SunRed™ Phosphate	N/A	653	661	• Most sensitive NIR phosphatase substrate
D-Luciferin phosphate *CAS 145613-12-3*	N/A	N/A	410	• Highly sensitive • Chemiluminescent substrate

* Absorption (Abs), fluorescence excitation (Ex) and fluorescence emission maxima (Em), in nanometers (nm). N/A = not applicable.

Concentration Curve

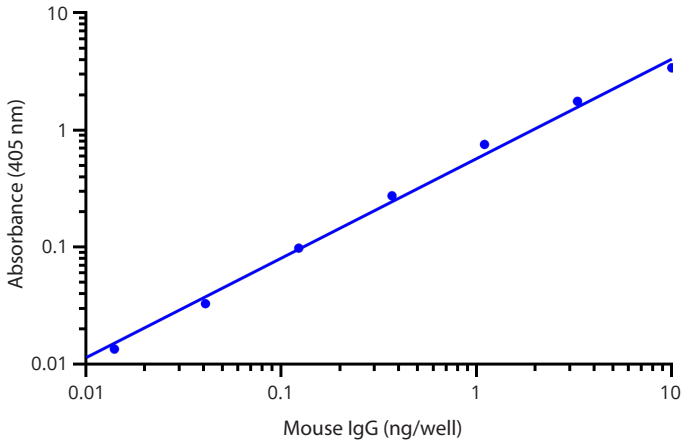


Figure 1. Detection of mouse IgG using goat anti-mouse IgG AP conjugates. Goat-anti mouse IgG antibody was labeled using Buccutite™ AP Antibody Conjugation Kit (Cat No. 5513). A 3-fold serial dilution of mouse IgG antibody was coated on a white wall/clear bottom 96-well plate. 100 µL goat anti-mouse IgG AP conjugate (1 µg/mL) was tested using the standard ELISA method. 100 µL pNPP substrate solution (Cat No. 11619) was added to the wells and allowed to develop for 30 minutes. Plate was read at 405 nm on an absorbance microplate reader.

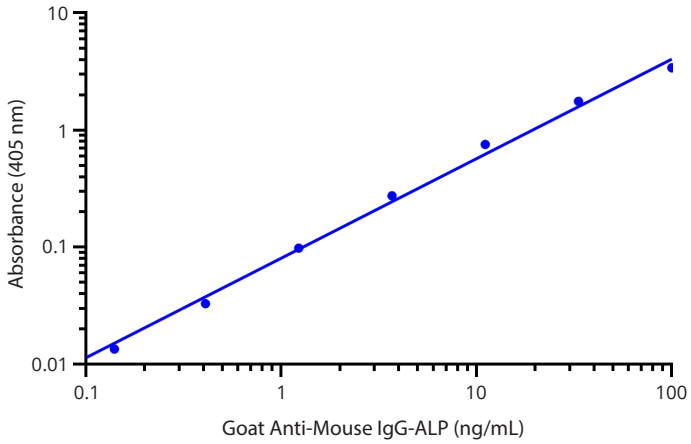


Figure 2. Detection of mouse IgG using goat anti-mouse IgG AP conjugates. Goat-anti mouse IgG antibody was labeled using Buccutite™ AP Antibody Conjugation Kit (Cat No. 5513). Mouse antibody (100 ng) was coated on a white wall/clear bottom 96-well plate. A 3-fold serial dilution of goat anti-mouse IgG AP conjugates was incubated and tested using the standard ELISA method. 100 µL pNPP substrate solution (Cat No. 11619) was added to the wells and allowed to develop for 30 minutes. Plate was read at 405 nm on an absorbance microplate reader.

Ordering Information

Cat No.	Product	Quantity
5512	Buccutite™ AP (Alkaline Phosphatase) Antibody Conjugation Kit *Optimized for Labeling 25 ug Protein*	1 kit
5513	Buccutite™ AP (Alkaline Phosphatase) Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*	1 kit
5514	Buccutite™ AP (Alkaline Phosphatase) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	1 kit
11627	DiFMUP	5 mg
12512	D-Luciferin phosphate	1 mg
11600	FDP [Fluorescein diphosphate, tetraammonium salt]	5 mg
11614	MUP [4-Methylumbelliferyl phosphate, free acid]	25 mg
11617	MUP [4-Methylumbelliferyl phosphate, free acid]	5 g
11610	MUP, disodium salt [4-Methylumbelliferyl phosphate, disodium salt]	25 mg
11612	MUP, disodium salt [4-Methylumbelliferyl phosphate, disodium salt]	10 g
11630	PhosLite™ Green	1 mg
11619	pNPP [4-Nitrophenyl phosphate, disodium salt]	25 mg
11622	Signal Guard™ phosphatase reaction stopping solution	100 mL
11629	SunRed™ Phosphate	5 mg

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with PE
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid PE Antibody Labeling Kits

The Buccutite™ Rapid PE Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with phycoerythrin (PE). To streamline the process, PE provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated PE component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Antibody conjugates formed using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid PE Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated PE
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

Phycoerythrin

Phycoerythrin is a 240 kDa phycobiliprotein isolated from red algae. It's spectral profile is characterized by absorbance maxima at 496 nm, 545 nm and 565 nm, and a single emission maximum at 574 nm. PE can be excited by blue, green or yellow laser lines and detected using a PE filter set (e.g. 575/30 nm or 585/40 nm). The sizeable molar extinction coefficient and quantum yield of PE makes it one of the brightest labels available for flow cytometry and cell sorting applications. Due to its rapid photobleaching characteristics, PE is not suitable for fluorescence microscopy.

Table 1. Spectral characteristiccs of PE.

Phycoerythrin	
Brightness Estimation	Very Bright
Molecular Weight	240,000 daltons
Ex (max)	496 nm, 545nm and 565 nm
Em (max)	574 nm
Extinction Coefficient	1,960,000 $cm^{-1}M^{-1}$
Quantum Yield	0.84
Stokes Shift from 496 nm / 545 nm / 565 nm	78 nm / 29 nm / 9 nm
Laser Lines	488 nm, 532 nm, 561-568 nm
Filter Set	585/40 nm

PE Spectrum

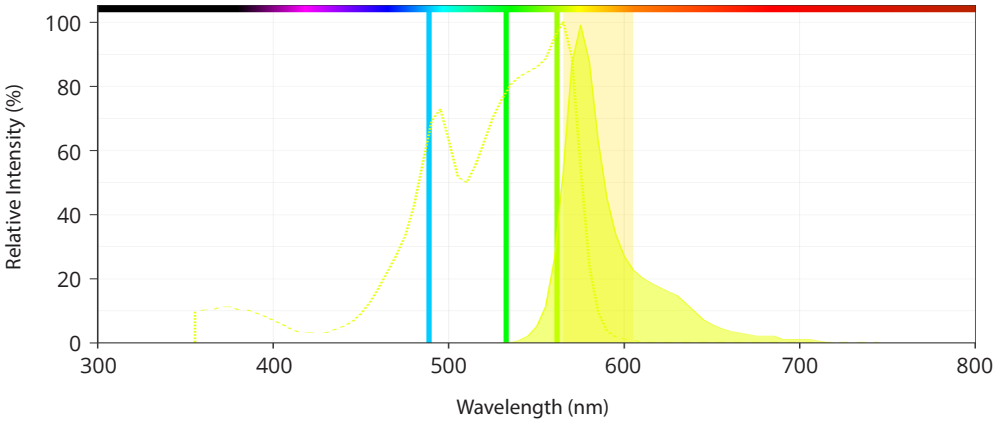


Figure 1. Excitation (yellow dotted line) and emission profile (yellow shaded region) of PE. PE can be excited by blue, green and yellow laser-equipped flow cytometers (488 nm, 532 nm, 561-568 nm) and detected in the 585/40 nm filter set.

PBMC Staining

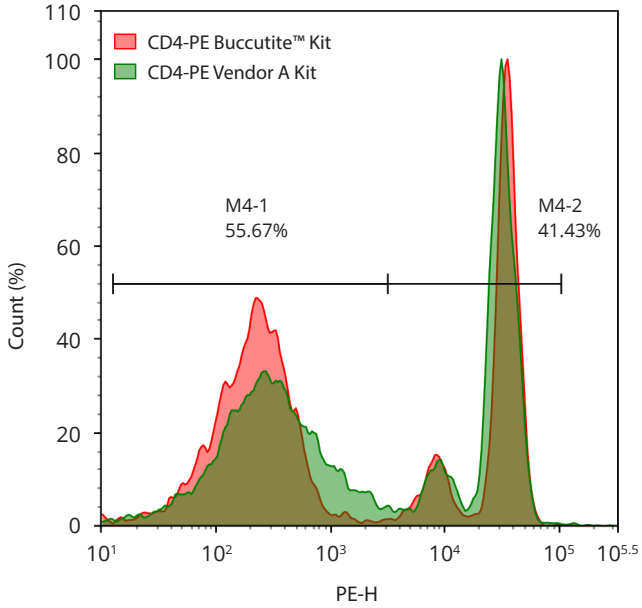


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid PE Antibody Labeling Kit (Cat No. 1310) or Vendor A Antibody Labeling Kit according to manufacturers' instructions. CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE channel.

Labeling Kit	Conjugate	Clone	Stain Index
Buccutite™ Kit	PE-CD4	SK3	55
Vendor A Kit	PE-CD4	SK3	28

Ordering Information

Cat No.	Product	Quantity
1312	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1310	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with PE-Texas Red tandem
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kits

The Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with PE-Texas Red. To streamline the process, PE-Texas Red provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated PE-Texas Red component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates produced using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months). Each kit includes sufficient reagents to perform two labeling reactions of either 25 µg (Cat No. 1343) or 100 µg (Cat No. 1318) of antibody.

Each Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated PE-Texas Red Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

PE-Texas Red Tandem

PE-Texas Red is a 240 kDa tandem fluorophore comprised of phycoerythrin (PE) and a Texas Red acceptor dye. It exhibits absorbance maxima at 496 nm, 545 nm and 565 nm, and a single emission maximum at 600 nm. PE-Texas Red can be excited by blue, green or yellow laser lines and detected using a Texas Red filter set (e.g. 610/20 nm). The emission profiles of PE-Texas Red and PE exhibit considerable spectral overlap. When used together, compensation is necessary to correct for spillover.

Table 1. Spectral characteristics of PE-Texas Red tandem.

PE-Texas Red	
Brightness Estimation	Bright
Molecular Weight	~240,000 daltons
Ex (max)	496 nm, 545nm and 565 nm
Em (max)	615 nm
Extinction Coefficient	1,960,000 $cm^{-1}M^{-1}$
Stokes Shift from 496 nm / 545 nm / 565 nm	119 nm / 70 nm / 50 nm
Laser Lines	488 nm, 532 nm, 561-568 nm
Filter Set	610/20 nm

PE-Texas Red Spectrum

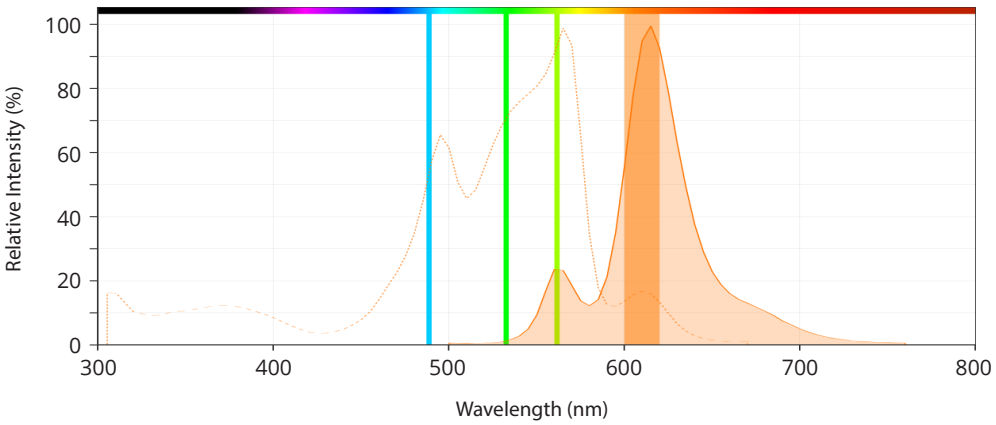


Figure 1. Excitation (orange dotted line) and emission profile (orange shaded region) of PE-Texas Red tandem dye. PE-Texas Red can be excited by blue, green or yellow laser-equipped flow cytometers (488 nm, 532 nm, 561-568 nm) and detected in the 610/20 nm filter set.

PBMC Staining

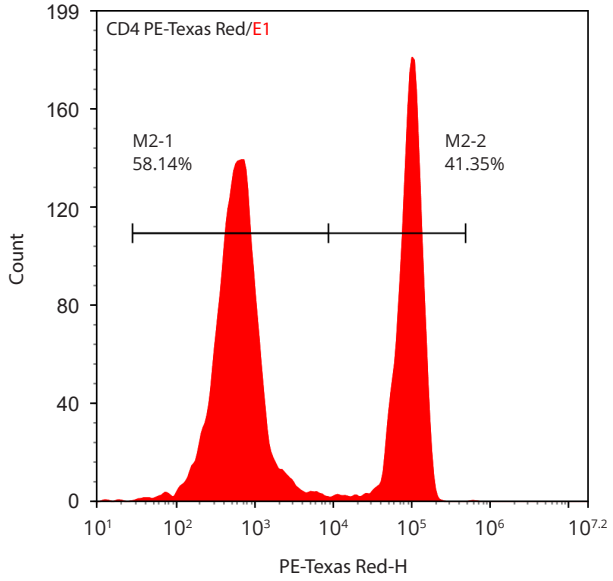


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit (Cat No. 1318). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Texas Red channel.

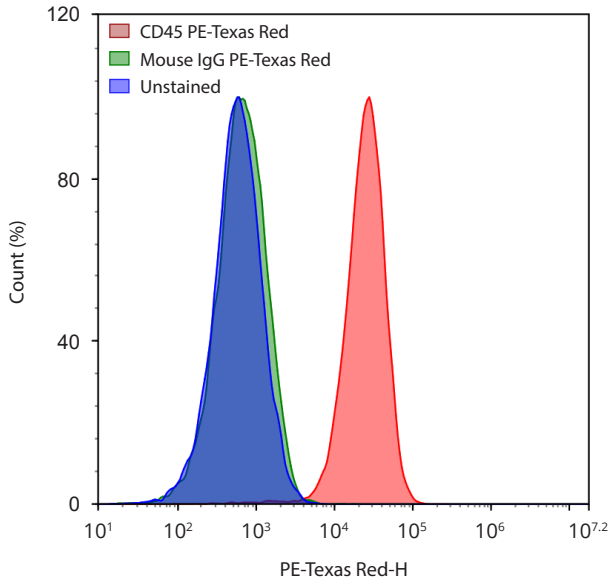


Figure 3. Flow cytometry analysis of CD45 PBMC populations. Anti-human CD45 monoclonal antibody and mouse IgG isotype control were labeled using Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit (Cat No. 1318). CD45 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Texas Red channel.

Ordering Information

Cat No.	Product	Quantity
1343	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with PE-Cy5 tandem
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kits

The Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with PE-Cy5. To streamline the process, PE-Cy5 provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated PE-Cy5 component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates produced using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated PE-Cy5 Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

PE-Cy5 Tandem

PE-Cy5 is a 240 kDa tandem fluorophore comprised of phycoerythrin (PE) and a Cy5 acceptor dye. It exhibits absorbance maxima at 496 nm, 545 nm and 565 nm, and a single emission maximum at 674 nm. PE-Cy5 can be excited by blue, green or yellow laser lines and detected using a Cy5 filter set (e.g. 670/40 nm, 675/20 nm or 695/40 nm). Attributed by a large Stokes Shift and long emission wavelength, PE-Cy5 is ideal for multicolor flow cytometry experiments.

Since PE-Cy5 shares an equivalent emission profile with APC, it is recommended that they are not used simultaneously. Furthermore, PE-Cy5 is sensitive to fixatives. When used to analyze intracellular markers be cautious of prolonged exposure as it can lead to the degradation of PE-Cy5.

Table 1. Spectral properties of PE-Cy5 tandem.

	PE-Cy5
Brightness Estimation	Very Bright
Molecular Weight	~240,000 daltons
Ex (max)	496 nm, 545nm and 565 nm
Em (max)	674 nm
Stokes Shift from 496 nm / 545 nm / 565 nm	178 nm / 129 nm / 109 nm
Extinction Coefficient	1,960,000 $cm^{-1}M^{-1}$
Laser Lines	488 nm, 532 nm, 561-568 nm
Filter set	670/40 nm

PE-Cy5 Spectrum

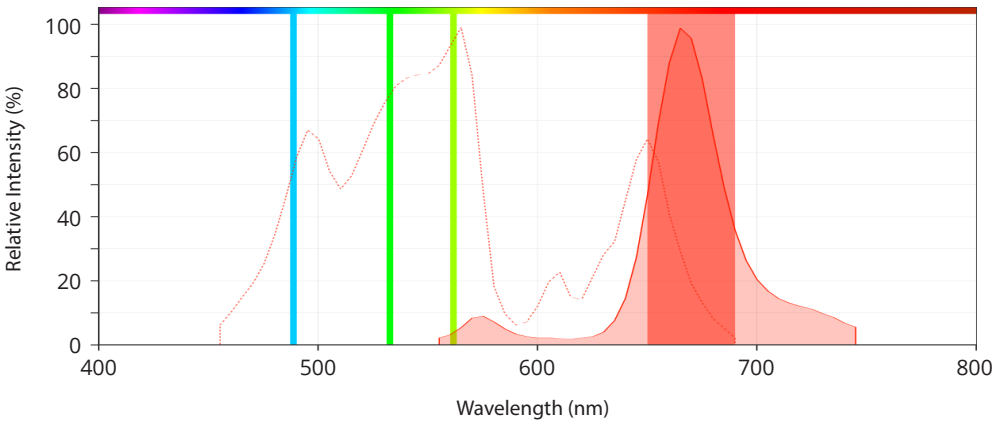


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of PE-Cy5 tandem dye. PE-Cy5 can be excited by blue, green or yellow laser-equipped flow cytometers (488 nm, 532 nm, 561-568 nm) and detected in the 670/40 nm filter set.

PBMC Staining

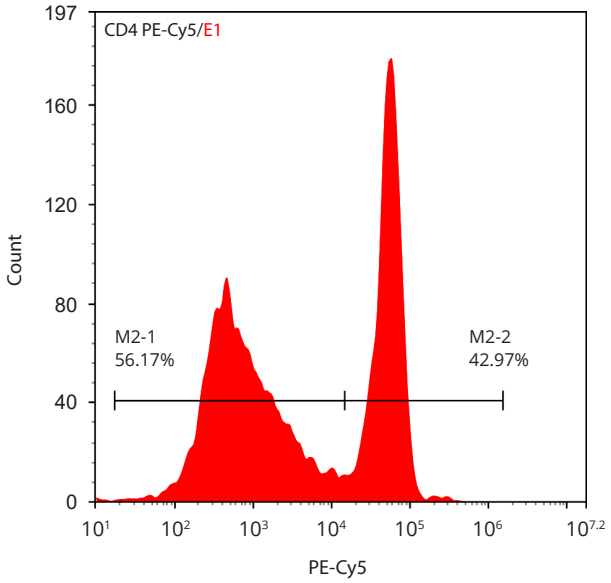


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit (Cat No. 1322). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Cy5 channel.

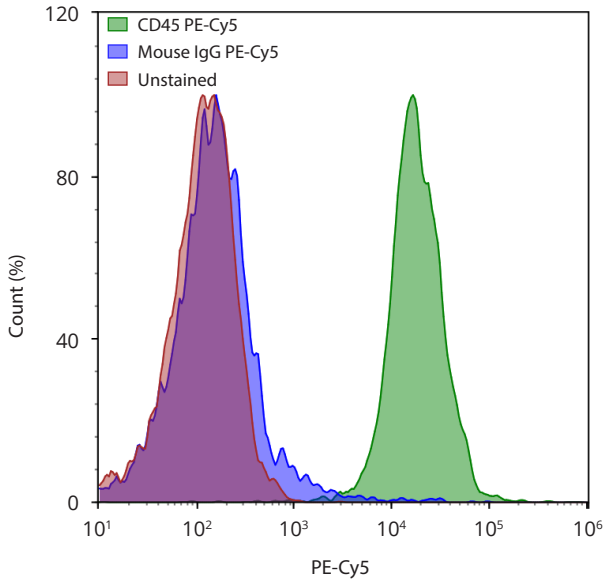


Figure 3. Flow cytometry analysis of CD45 PBMC populations. Anti-human CD45 monoclonal antibody and mouse IgG isotype control were labeled using Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit (Cat No. 1322). CD45 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Cy5 channel.

Ordering Information

Cat No.	Product	Quantity
1340	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1322	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with PE-Cy5.5 tandem
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kits

The Buccutite™ Rapid PE-Cy5.5 Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with PE-Cy5.5. To streamline the process, PE-Cy5.5 provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated PE-Cy5.5 component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates produced using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated PE-Cy5.5 Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

PE-Cy5.5 Tandem

PE-Cy5.5 is a 240 kDa tandem fluorophore comprised of phycoerythrin (PE) and a Cy5.5 acceptor dye. It exhibits absorbance maxima at 496 nm, 545 nm and 565 nm, and a single emission maximum at 700 nm. PE-Cy5.5 can be excited by blue, green or yellow laser lines and detected using a Cy5.5 filter set (e.g. 682/33 nm or 695/40 nm). Attributed by a large Stokes Shift and long emission wavelength, PE-Cy5.5 is ideal for multicolor flow cytometry experiments.

Table 1. Spectral properties of PE-Cy5.5 tandem.

PE-Cy5.5	
Brightness Estimation	Bright
Molecular Weight	~240,000 daltons
Ex (max)	496 nm, 545nm and 565 nm
Em (max)	700 nm
Extinction Coefficient	1,960,000 $cm^{-1}M^{-1}$
Stokes Shift from 496 nm / 545 nm / 565 nm	204 nm / 155 nm / 135 nm
Laser Lines	488 nm, 532 nm, 561-568 nm
Filter sets	682/33 nm or 695/40 nm

PE-Cy5.5 Spectrum

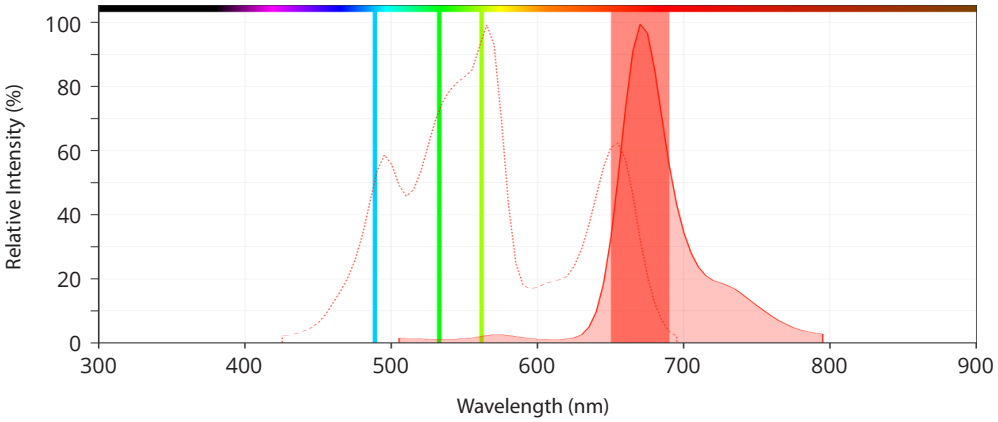


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of PE-Cy5.5 tandem dye. PE-Cy5.5 can be excited by blue, green or yellow laser-equipped flow cytometers (488 nm, 532 nm, 561-568 nm) and detected in the 682/33 nm filter set.

PBMC Staining

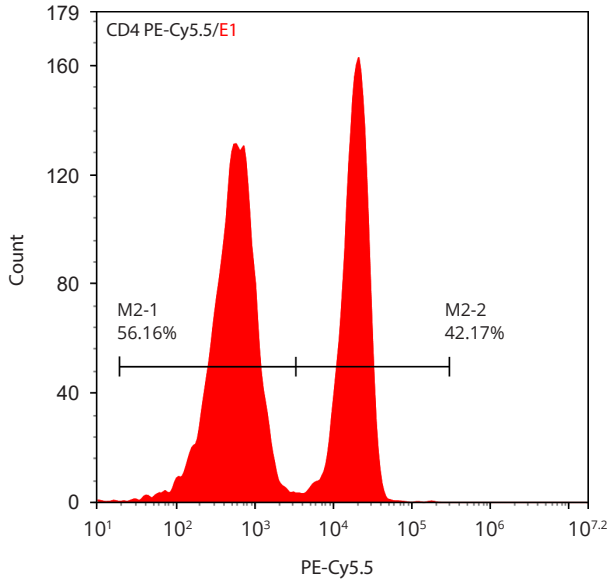


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit (Cat No. 1316). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Cy5.5 channel.

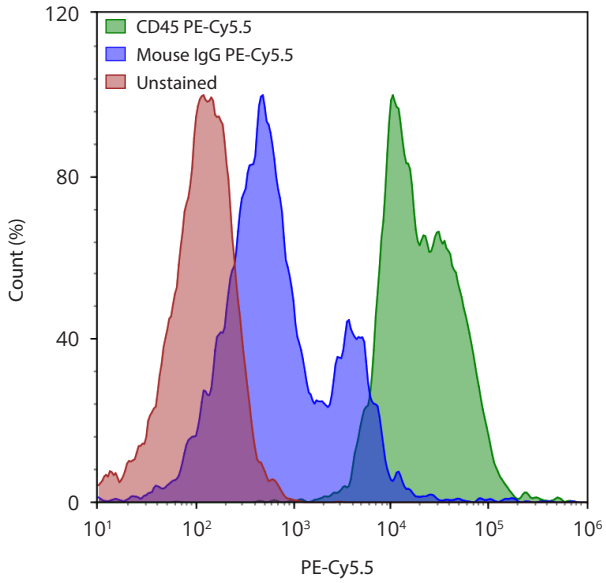


Figure 3. Flow cytometry analysis of CD45 PBMC populations. Anti-human CD45 monoclonal antibody and mouse IgG isotype control were labeled using Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit (Cat No. 1316). CD45 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Cy5.5 channel.

Ordering Information

Cat No.	Product	Quantity
1341	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kits

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with PE-Cy7 tandem
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

The Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with PE-Cy7. To streamline the process, PE-Cy7 provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated PE-Cy7 component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates formed using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated PE-Cy7 Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

PE-Cy7 Tandem

PE-Cy7 is a 240 kDa tandem fluorophore comprised of phycoerythrin (PE) and a Cy7 acceptor dye. It exhibits absorbance maxima at 496 nm, 545 nm and 565 nm, and a single emission maximum at 780 nm. PE-Cy7 can be excited by blue, green or yellow laser lines and detected using a Cy7 filter set (e.g. 780/60 nm or 755 LP). Attributed by a large Stokes Shift and long emission wavelength, PE-Cy7 is ideal for multicolor flow cytometry experiments.

Compared to PE-Cy5, PE-Cy7 is highly sensitive to fixatives and photo-induced degradation resulting in significant loss of fluorescence. When used to analyze intracellular markers be cautious of prolonged exposure as it can also lead to the degradation of PE-Cy7. It is recommended that fixed cells stained with PE-Cy7 antibody conjugates be analyzed within 4 hours of fixation in paraformaldehyde.

Table 1. Spectral properties of PE-Cy7 tandem.

PE-Cy7 Tandem	
Brightness Estimation	Bright
Molecular Weight	~240,000 daltons
Ex (max)	496 nm, 545nm and 565 nm
Em (max)	780 nm
Stokes Shift from 565 nm	215 nm
Extinction Coefficient	1,960,000 $cm^{-1}M^{-1}$
Laser Lines	488 nm, 532 nm, 561-568 nm
Filter Set	780/60 nm

PE-Cy7 Spectrum

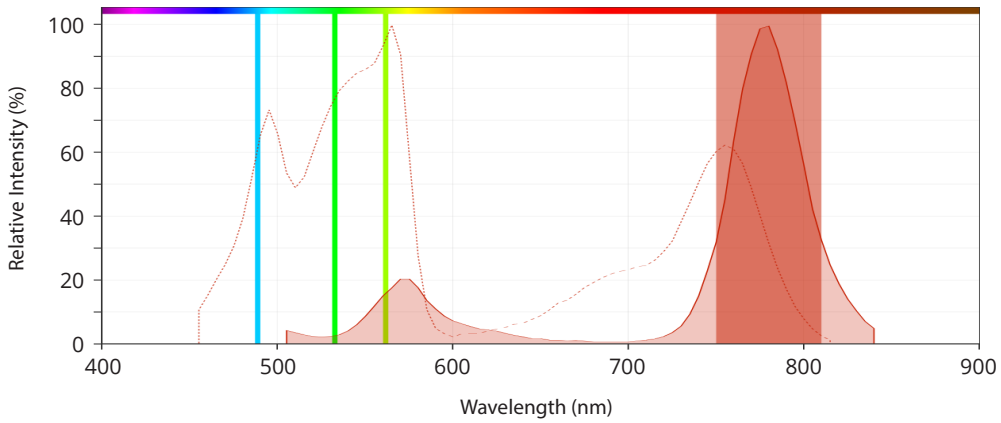


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of PE-Cy7 tandem dye. PE-Cy7 can be excited by blue, green or yellow laser-equipped flow cytometers (488 nm, 532 nm, 561-568 nm) and detected in the 780/60 nm filter set.

PBMC Staining

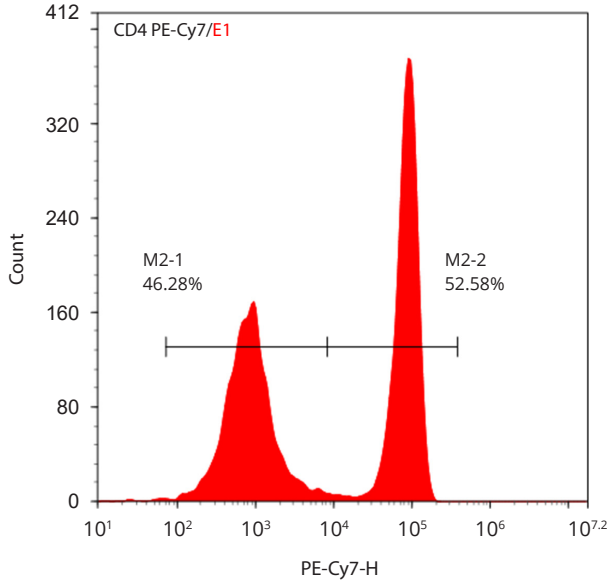


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit (Cat No. 1317). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Cy7 channel.

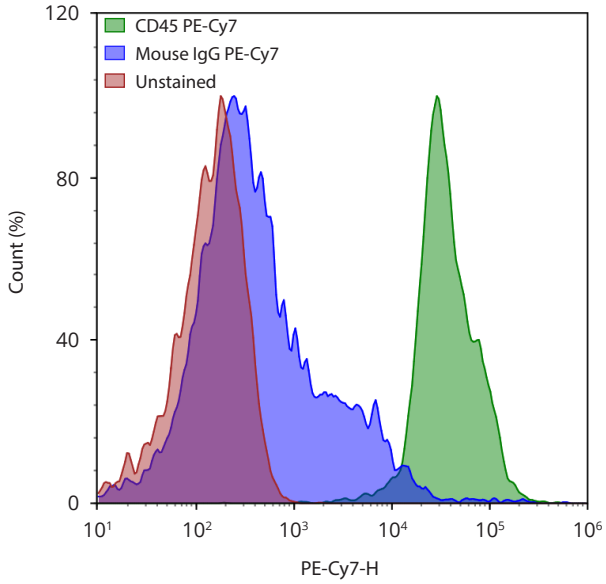


Figure 3. Flow cytometry analysis of CD45 PBMC populations. Anti-human CD45 monoclonal antibody and mouse IgG isotype control were labeled using Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit (Cat No. 1317). CD45 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PE-Cy7 channel.

Ordering Information

Cat No.	Product	Quantity
1342	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with APC
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid APC Antibody Labeling Kits

The Buccutite™ Rapid APC Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with APC. To streamline the process, APC provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated APC component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates formed using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid APC Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated APC
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

Allophycocyanin

Allophycocyanin (APC) is a 105 kDa phycobiliprotein isolated from *Spirulina sp.*, a blue-green algae. It exhibits absorbance maxima at 625 nm and 651 nm, and a single emission maximum at 662 nm. APC can be excited by the red laser lines and detected using an APC filter set (e.g. 660/20 nm). The sizeable molar extinction coefficient and quantum yield of APC makes it an exceptionally bright label for flow cytometry and cell sorting applications. Since APC shares an equivalent spectral profile with dyes such as iFluor™ 647 and Alexa Fluor® 647 it is recommended that they are not used simultaneously.

Although not as optimal as a red laser, APC can be excited by the 561 nm laser. However, in cells simultaneously stained with APC and PE-Cy5 conjugates excitation by the 561 nm laser will cause significant spillover into the PE-Cy5 channel. To account for such artifacts considerable crossbeam compensation must be performed to subtract APC fluorescence from the PE-Cy5 signal.

Table 1. Spectral properties of APC.

Allophycocyanin	
Brightness Estimation	Very Bright
Molecular Weight	105,000 daltons
Ex (max)	651 nm
Em (max)	662 nm
Extinction Coefficient	700,000 $cm^{-1}M^{-1}$
Quantum Yield	0.68
Stokes Shift	11 nm
Laser Lines	633-647 nm
Filter set	660/20 nm

APC Spectrum

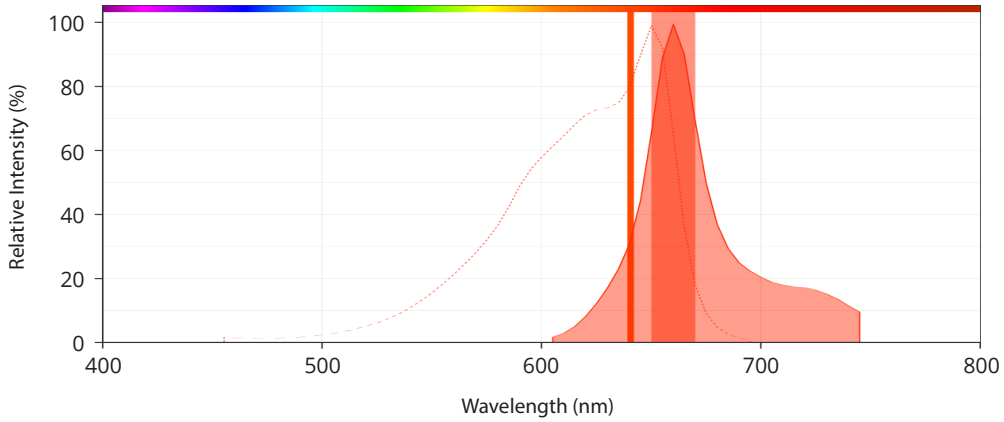


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of APC. APC can be excited by red laser-equipped flow cytometers (633-640 nm) and detected in the 660/20 nm filter set.

PBMC Staining

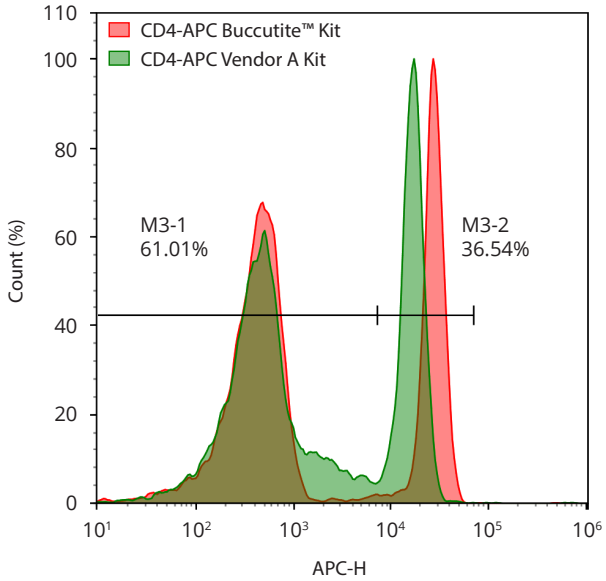


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid APC Antibody Labeling Kit (Cat No. 1313) or Lightning-Link® Rapid APC Antibody Labeling Kit according to manufacturers' instructions. CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the APC channel.

Labeling Kit	Conjugate	Clone	Stain Index
Buccutite™ Kit	APC-CD4	SK3	24
Vendor A Kit	APC-CD4	SK3	8

Ordering Information

Cat No.	Product	Quantity
1313	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1311	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with APC-Cy5.5 tandem
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kits

The Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with APC-Cy5.5. To streamline the process, APC-Cy5.5 provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated APC-Cy5.5 component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates formed using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated APC-Cy5.5 Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

APC-Cy5.5 Tandem

APC-Cy5.5 is a 105 kDa tandem fluorophore comprised of allophycocyanin (APC) and a Cy5.5 acceptor dye. It exhibits absorbance maxima at 625 nm and 651 nm, and a single emission maximum at 700 nm. APC-Cy5.5 can be excited by the red laser lines and detected using a Cy5.5 filter set (e.g. 695/40 nm).

Although not as optimal as a red laser, APC-Cy5.5 can be excited by the 561 nm laser. However, in cells simultaneously stained with APC-Cy5.5 and PE-Cy5.5 conjugates excitation by the 561 nm laser will cause significant spillover into the PE-Cy5.5 channel. To account for such artifacts considerable crossbeam compensation must be performed to subtract APC-Cy5.5 fluorescence from the PE-Cy5.5 signal.

Table 1. Spectral properties of APC-Cy5.5 tandem.

APC-Cy5.5 Tandem	
Brightness Estimation	Dim
Molecular Weight	~105,000 daltons
Ex (max)	651 nm
Em (max)	700 nm
Stokes Shift	49 nm
Extinction Coefficient	700,000 $cm^{-1}M^{-1}$
Laser Lines	633-647 nm
Filter Set	695/40 nm

APC-Cy5.5 Spectrum

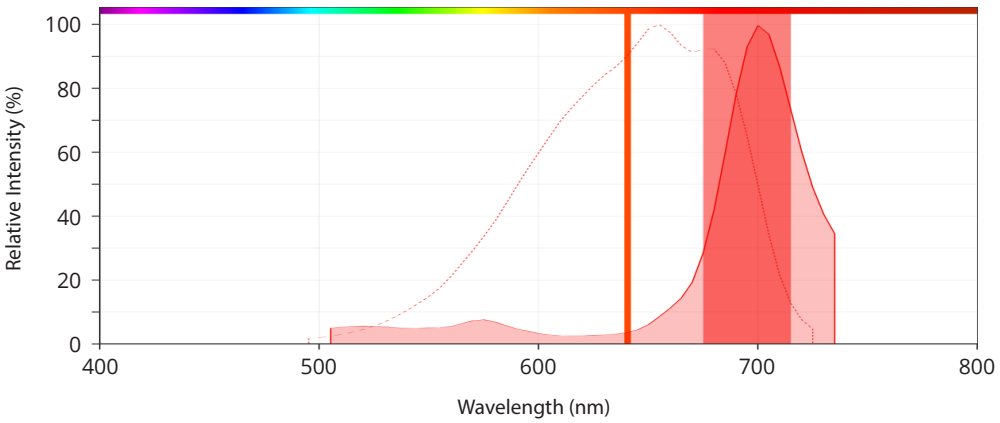


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of APC-Cy5.5 tandem dye. APC-Cy5.5 can be excited by red laser-equipped flow cytometers (633-640 nm) and detected in the 695/40 nm filter set.

PBMC Staining

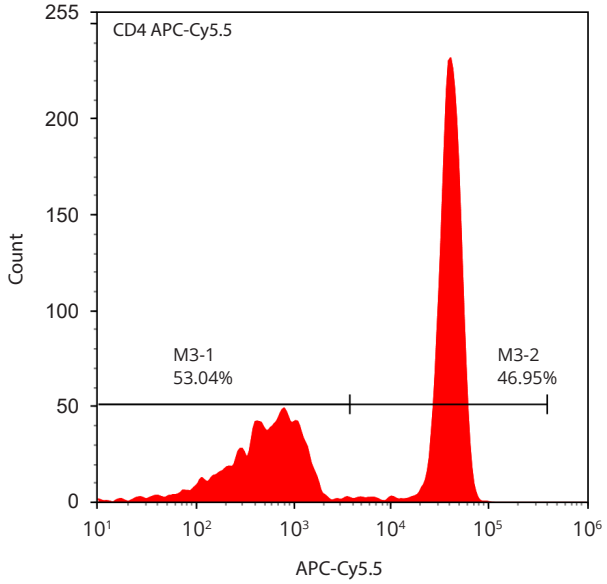


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit (Cat No. 1320). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the APC-Cy5.5 channel.

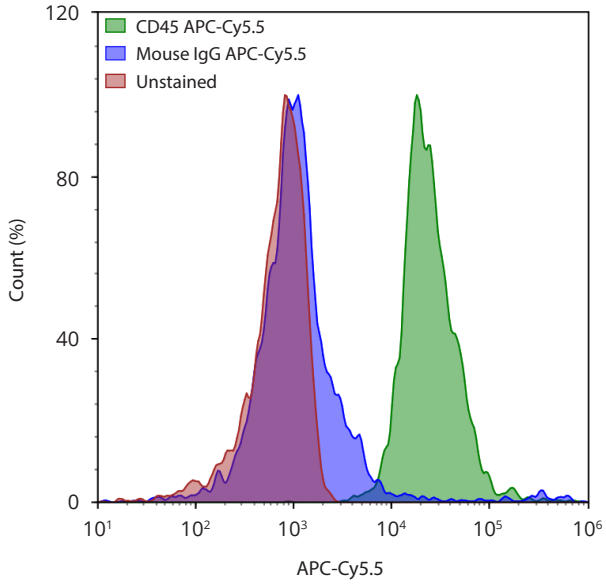


Figure 3. Flow cytometry analysis of CD45 PBMC populations. Anti-human CD45 monoclonal antibody and mouse IgG isotype control were labeled using Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit (Cat No. 1320). CD45 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the APC-Cy5.5 channel.

Ordering Information

Cat No.	Product	Quantity
1350	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1320	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with APC-iFluor™ 700 tandem
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kits

Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with APC-iFluor™ 700. To streamline the process, APC-iFluor™ 700 provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated APC-iFluor™ 700 component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates formed using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated APC-iFluor™ 700 Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

APC-iFluor™ 700 Tandem

APC-iFluor™ 700 is a 105 kDa tandem fluorophore comprised of allophycocyanin (APC) and an iFluor™ 700 acceptor dye. It exhibits a primary absorption maximum at 651 nm, a secondary absorption maximum at 625 nm, and a single emission maximum of 713 nm. APC-iFluor™ 700 can be excited by the red laser and detected using a Cy5.5 filter (e.g. 695/40 nm and 730/45 nm). Since APC-iFluor™ 700 shares an nearly identical spectral profile with APC-Cy5.5 they cannot be used simultaneously. For multicolor flow cytometric analysis using a single excitation source use APC-iFluor™700 in conjunction with iFluor™ 647 or Alexa Fluor® 647, and APC-Cy7 conjugates.

Although not as optimal as a red laser, APC-iFluor™700 can be excited by the 561 nm laser. However, in cells simultaneously stained with APC-iFluor™ 700 and PE-Cy5.5 conjugates excitation by the 561 nm laser will cause significant spillover into the PE-Cy5.5 channel. To account for such artifacts considerable crossbeam compensation must be performed to subtract APC-iFluor™ 700 fluorescence from the PE-Cy5.5 signal.

Table 1. Spectral properties of APC-iFluor™ 700 tandem.

APC-iFluor™ 700 Tandem	
Brightness Estimation	Dim
Molecular Weight	~105,000 daltons
Ex (max)	651 nm
Em (max)	713 nm
Stokes Shift	62 nm
Extinction Coefficient	700,000 $cm^{-1}M^{-1}$
Laser Lines	633-647 nm
Filter Set	720/30 nm

APC-iFluor™ 700 Spectrum

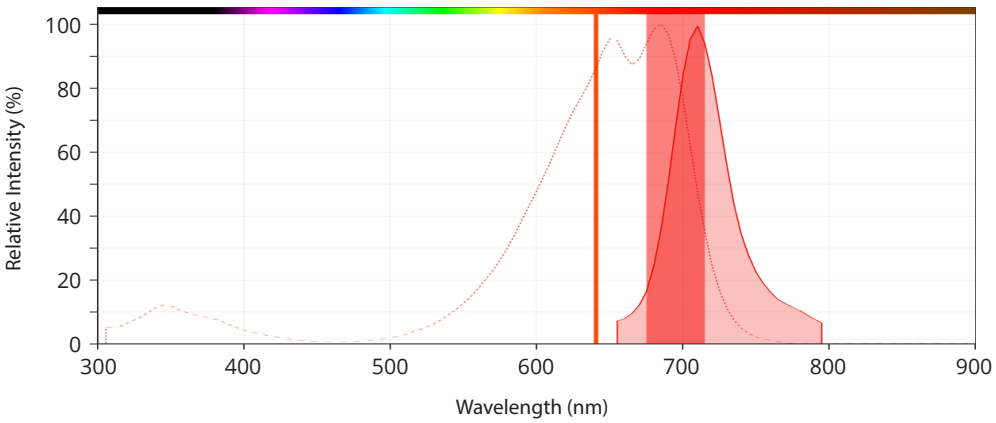


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of APC-iFluor™ 700 tandem dye. APC-iFluor™ 700 can be excited by red laser-equipped flow cytometers (633-640 nm) and detected in the 695/40 nm filter set.

PBMC Staining

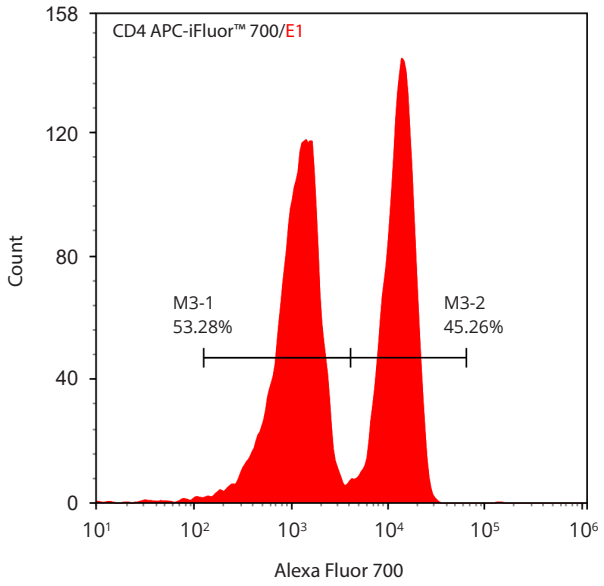


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit (Cat No. 1319). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the Alexa Fluor 700 channel.

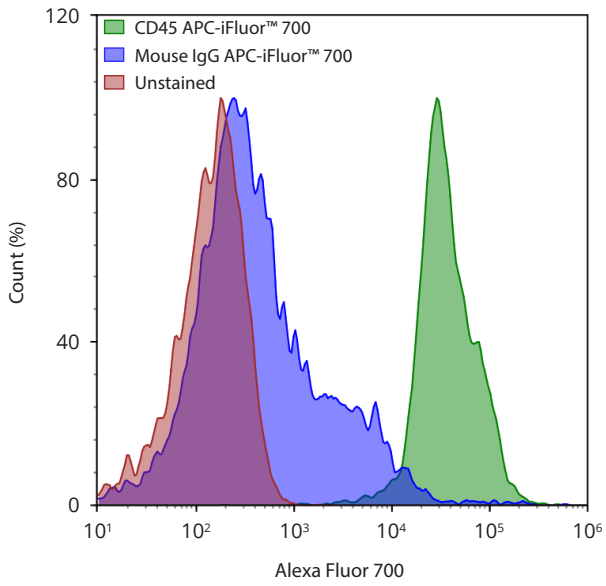


Figure 3. Flow cytometry analysis of CD45 PBMC populations. Anti-human CD45 monoclonal antibody and mouse IgG isotype control were labeled using Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit (Cat No. 1319). CD45 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the Alexa Fluor 700 channel.

Ordering Information

Cat No.	Product	Quantity
1347	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1319	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with APC-Cy7 tandem
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kits

The Buccutite™ Rapid APC-Cy7 Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with APC-Cy7. To streamline the process, APC-Cy7 provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated APC-Cy7 component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates formed using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated APC-Cy7 Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

APC-Cy7 Tandem

APC-Cy7 is a 105 kDa tandem fluorophore comprised of allophycocyanin (APC) and a Cy7 acceptor dye. It exhibits absorbance maxima at 625 nm and 651 nm, and a single emission maximum at 780 nm. APC-Cy7 can be excited by the red laser lines and detected using a Cy7 filter set (e.g. 780/60 or 755 LP nm). Of all the tandem dyes excited by the red laser line, APC-Cy7 has the longest wavelength emission.

Although not as optimal as a red laser, APC-Cy7 can be excited by the 561 nm laser. However, in cells simultaneously stained with APC-Cy7 and PE-Cy7 conjugates excitation by the 561 nm laser will cause significant spillover into the PE-Cy7 channel. To account for such artifacts considerable crossbeam compensation must be performed to subtract APC-Cy7 fluorescence from the PE-Cy7 signal.

APC-Cy7 is highly sensitive to fixatives and photo-induced degradation resulting in significant loss of fluorescence. When used to analyze intracellular markers be cautious of prolonged exposure as it can also lead to the degradation of APC-Cy7. It is recommended that fixed cells stained with APC-Cy7 antibody conjugates be analyzed within 4 hours of fixation in paraformaldehyde.

Table 1. Spectral properties of APC-Cy7 tandem.

APC-Cy7 Tandem	
Brightness Estimation	Dim
Molecular Weight	~105,000 daltons
Ex (max)	651 nm
Em (max)	780 nm
Stokes Shift	129 nm
Extinction Coefficient	700,000 $cm^{-1}M^{-1}$
Laser Lines	633-647 nm
Filter Set	780/60 nm

APC-Cy7 Spectrum

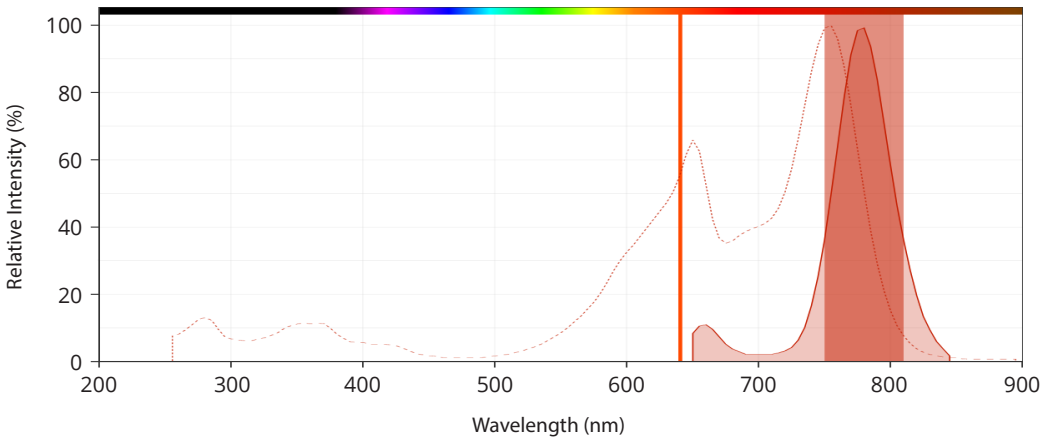


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of APC-Cy7 tandem dye. APC-Cy7 can be excited by red laser-equipped flow cytometers (633-640 nm) and detected in the 780/60 nm filter set.

PBMC Staining

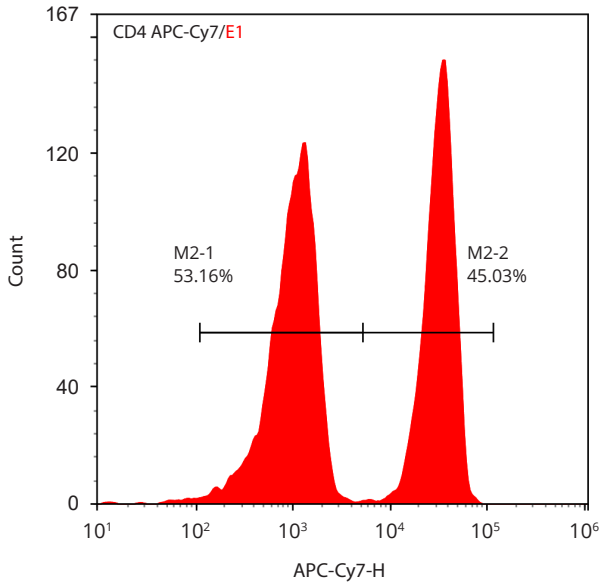


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit (Cat No. 1321). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the APC-Cy7 channel.

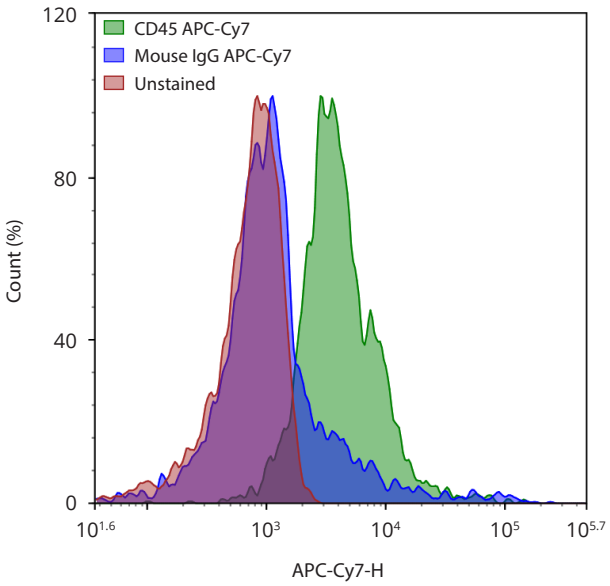


Figure 3. Flow cytometry analysis of CD45 PBMC populations. Anti-human CD45 monoclonal antibody and mouse IgG isotype control were labeled using Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit (Cat No. 1321). CD45 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the APC-Cy7 channel.

Ordering Information

Cat No.	Product	Quantity
1351	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1321	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Key Features

- Efficient method for covalently labeling 25 to 100 µg of antibody with PerCP
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Excellent batch-to-batch reproducibility
- No column purification step required
- 100% recovery of materials
- Conjugates stable for long-term storage (4 °C, 12 months)
- Conjugates can be used for fluorescence activated cell sorting, multicolor flow cytometry and immunophenotyping

Buccutite™ Rapid PerCP Antibody Labeling Kits

The Buccutite™ Rapid PerCP Antibody Labeling Kits offer a quick and convenient method to covalently label primary antibodies with PerCP. To streamline the process, PerCP provided in these kits come preactivated with Buccutite™ FOL and can be directly used for antibody conjugation. Simply activate the desired antibody or protein to label with Buccutite™ MTA and then mix together with the Buccutite™ FOL-Activated PerCP component. Conjugation is robust taking two hours or less to complete, with minimal batch-to-batch variation, no separation step and 100% recovery of antibody. Conjugates formed using Buccutite™ technology are ideal for multicolor flow cytometry, immunophenotyping or cell sorting applications, and are highly stable for long-term storage (4°C, 12 months).

Each Buccutite™ Rapid PerCP Antibody Labeling Kit provides sufficient reagents to perform 2 separate labeling reactions of 25 µg or 100 µg of antibody each, including:

- Buccutite™ FOL-Activated APC-Cy7 Tandem
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Detailed protocol for conjugation

Peridinin-Chlorophyll-Protein Complex

Peridinin-chlorophyll-protein complex (PerCP) is a 35.5 kDa fluorescent protein complex isolated from dinoflagellates. It exhibits a broad absorption spectrum with an excitation maximum at 477 nm and an emission maximum at 678 nm. PerCP can be excited by the blue laser and detected using a Cy5 filter set (e.g. 682/33 nm, 670/40 nm or 695/40 nm). Due to its rapid photobleaching characteristics, avoid using PerCP conjugates on flow cytometers equipped with high-power lasers (e.g. water-cooled gas lasers).

Table 1. Spectral properties of PerCP.

	PerCP
Brightness Estimation	Dim
Molecular Weight	35,000 daltons
Ex (max)	477 nm
Em (max)	678 nm
Stokes Shift	201 nm
Extinction Coefficient	350,000 $cm^{-1}M^{-1}$
Laser Lines	488 nm
Filter set	695/40 nm

PerCP Spectrum

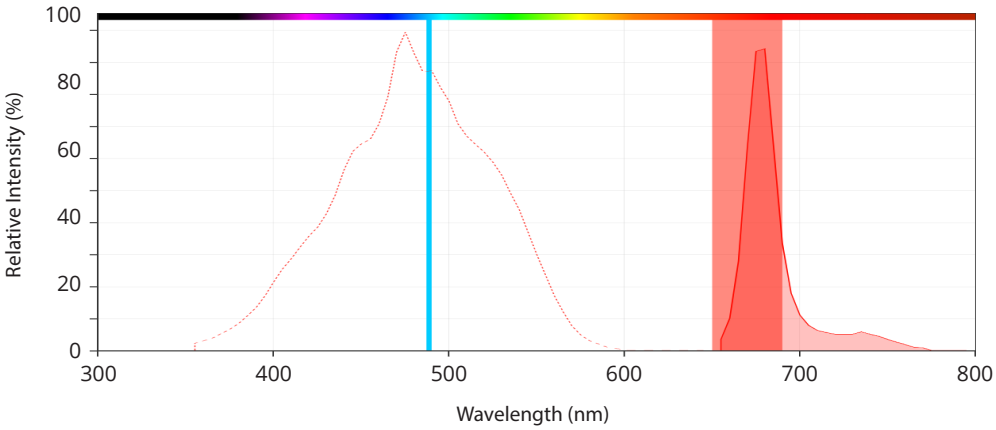


Figure 1. Excitation (red dotted line) and emission profile (red shaded region) of PerCP. PerCP can be excited by blue laser-equipped flow cytometers (488 nm) and detected in the 695/40 nm filter set.

PBMC Staining

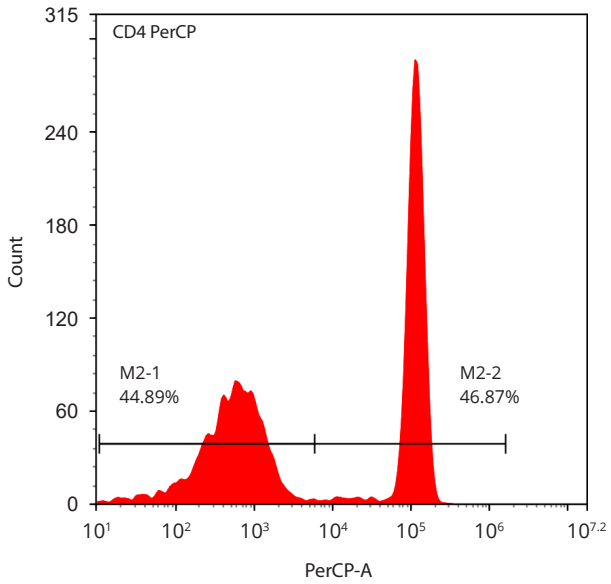


Figure 2. Flow cytometry analysis of CD4 PBMC populations. Anti-human CD4 monoclonal antibody was labeled using Buccutite™ Rapid PerCP Antibody Labeling Kit (Cat No. 1325). CD4 PBMC populations were then stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PerCP channel.

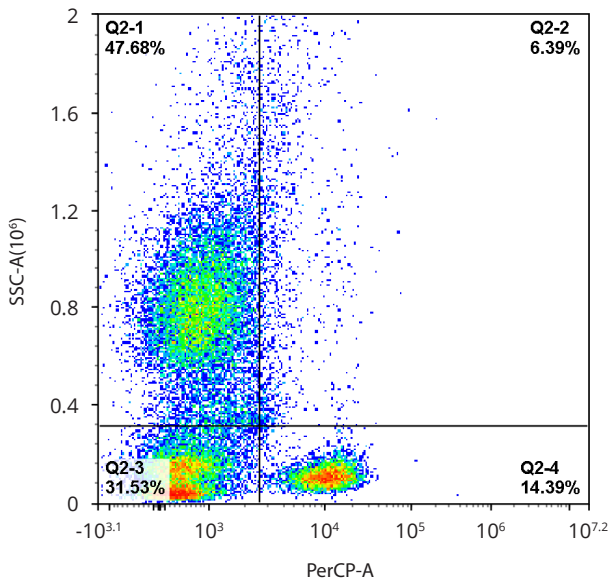


Figure 3. Flow cytometry analysis of CD3 PBMC populations. Anti-human CD3 monoclonal antibody was labeled using Buccutite™ Rapid PerCP Antibody Labeling Kit (Cat No. 1325). CD3 PBMC populations were than stained and the fluorescence signal was monitored using an ACEA NovoCyte flow cytometer in the PerCP channel.

Ordering Information

Cat No.	Product	Quantity
1353	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1325	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Buccutite™ Rapid Protein Crosslinking Kit

Key Features

- Efficient method for covalently labeling 100 µg of antibody with 100 µg of any protein greater than 25 kDa
- Conjugation can be performed in a wide range of pH 5.0-9.0
- Two Labeling reactions per kit
- Typical conjugation yield is 50-60%
- ~15 minues actual hands-on time
- Requires the removal of BSA, amine-containing molecules and other proteins stabilizers prior to labeling
- Requires a separation step prior to conjugate use
- Conjugates stable for long-term storage (4 °C, 12 months)

The Buccutite™ Rapid Protein Crosslinking Kit offers a quick and convenient method for covalently labeling any two purified proteins (> 25 kDa) for their subsequent use as probes in various downstream applications. Compared to the conventional SMCC technique, which is a troublesome and low-yielding process, the Buccutite™ Rapid Protein Crosslinking Kit is significantly more robust and less demanding. Conjugation can be completed in two hours or less, with minimal hands-on time and a high conjugation yield.

To conjugate two proteins, Buccutite™ Rapid Protein Crosslinking Kits use a pair of proprietary crosslinkers – Buccutite™ MTA and Buccutite™ FOL. Each crosslinker is independently labeled onto an antibody or protein of interest via its amine-reactive moiety. When mixed together, proteins will bind with a high degree of affinity and specificity to its Buccutite™ MTA/FOL counterpart. The covalent bond formed between the Buccutite™ linkers is extremely stable resulting in conjugates that can tolerate the rigorous washing process in a typical immunoassay (e.g. ELISA, IHC and Western blot) or flow cytometric application. This kit provides sufficient reagents to perform 2 separate labeling reactions of 100 µg of proteins each, including:

- Buccutite™ FOL
- Buccutite™ MTA
- Buccutite™ Reaction Buffer
- Spin Columns
- Detailed protocol for conjugation

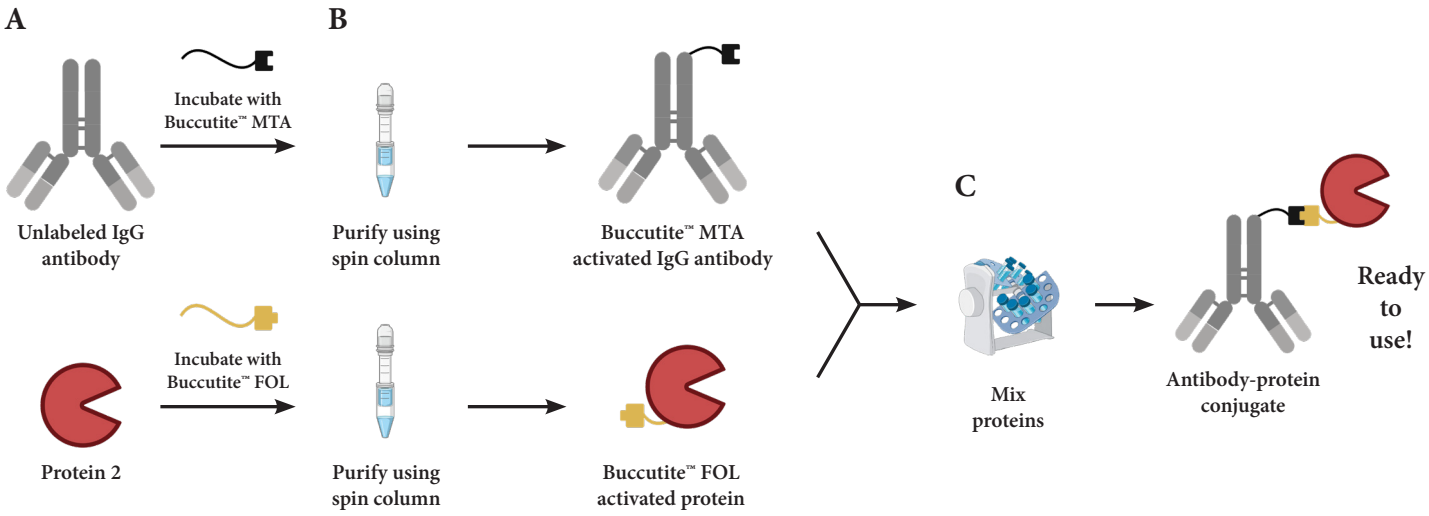


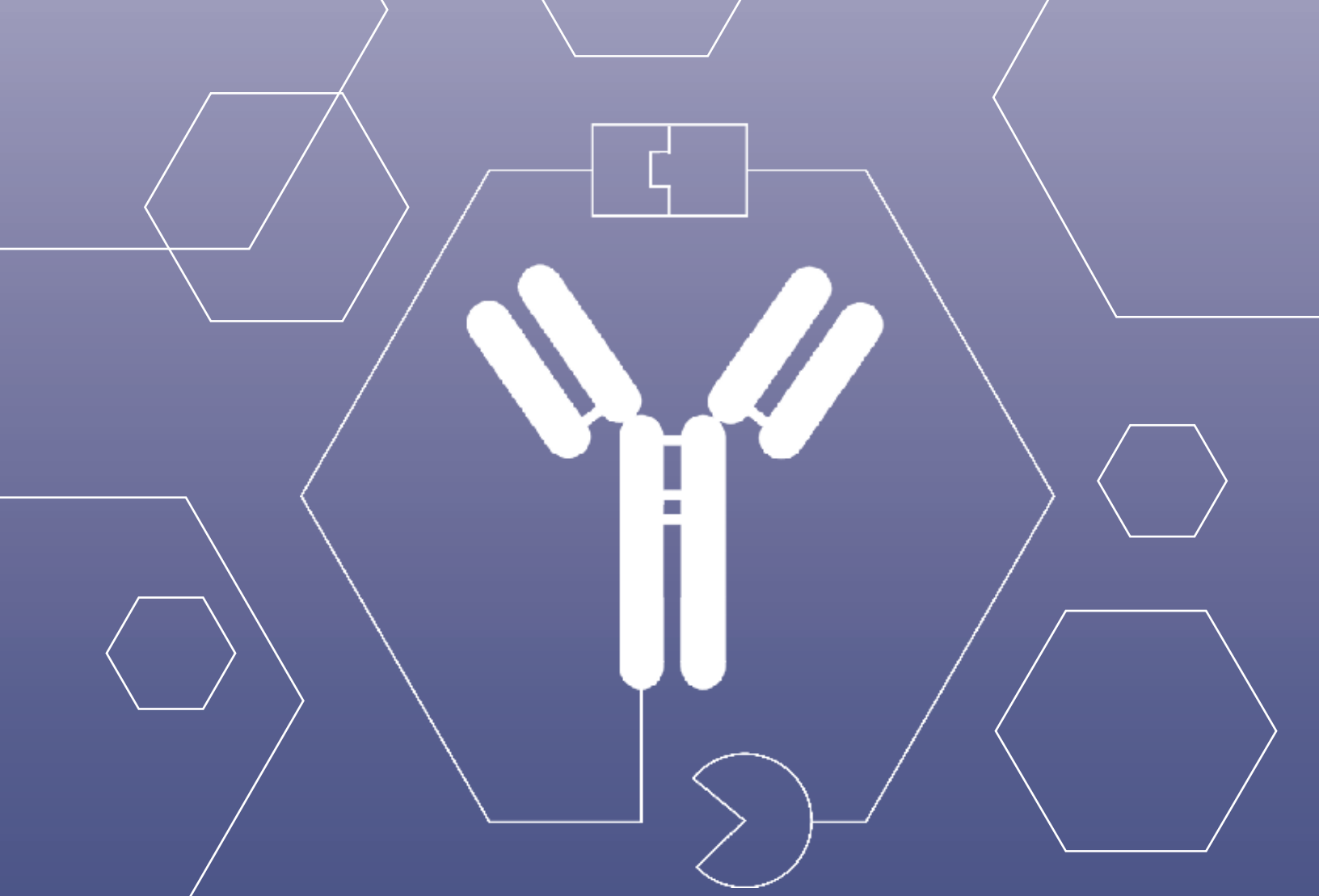
Figure 1. Schematic representation of the Buccutite™ Rapid Protein Crosslinking Kit. **A)** An unlabeled IgG antibody is incubated with the Buccutite™ MTA reagent and the protein it will be conjugated with is incubated with the Buccutite™ FOL reagent. **B)** Separately load Buccutite™ MTA and Buccutite™ FOL activated proteins into spin columns to purify. **C)** Crosslink proteins by mixing Buccutite™ activated proteins together at the desired molar ratios and rotate the mixture at room temperature for 1 hour.

Ordering Information

Cat No.	Product	Quantity
1315	Buccutite™ Rapid Protein Crosslinking Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings

Product List: Kits for Labeling Antiobdies & Proteins; AP Substrates & Reagents; HRP Substrates & Reagents

Cat No.	Product	Quantity
11000	Amplite™ ADHP [10-Acetyl-3,7-dihydroxyphenoxazine] *CAS#: 119171-73-2*	25 mg
11005	Amplite™ Blue	25 mg
11011	Amplite™ IR	1 mg
11011	Amplite™ Red	1000 Assays
5512	Buccutite™ AP (Alkaline Phosphatase) Antibody Conjugation Kit *Optimized for Labeling 25 ug Protein*	2 x 25 µg
5513	Buccutite™ AP (Alkaline Phosphatase) Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*	2 x 100 µg
5514	Buccutite™ AP (Alkaline Phosphatase) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	1 x 1 mg
5505	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 25 ug Protein*	2 x 25 µg
5503	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*	2 x 100 µg
5504	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	1 x 1 mg
5506	Buccutite™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	5 x 1 mg
5518	Buccutite™ Poly-HRP Antibody Conjugation Kit *Optimized for Labeling 50 µg Protein*	1 x 50 µg
5519	Buccutite™ Poly-HRP Antibody Conjugation Kit *Optimized for Labeling 50 µg Protein*	2 x 50 µg
1313	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1311	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1350	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1320	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1351	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1321	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1347	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1319	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1312	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1310	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1340	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1322	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1341	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1342	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1343	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1353	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	2 Labelings
1325	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
1315	Buccutite™ Rapid Protein Crosslinking Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	2 Labelings
11627	DiFMUP	5 mg
12512	D-Luciferin phosphate	1 mg
11600	FDP [Fluorescein diphosphate, tetraammonium salt]	5 mg
11050	Luminol [3-Aminophthalhydrazide] *CAS 521-31-3*	1 g
11614	MUP [4-Methylumbelliferyl phosphate, free acid]	25 mg
11617	MUP [4-Methylumbelliferyl phosphate, free acid]	5 g
11610	MUP, disodium salt [4-Methylumbelliferyl phosphate, disodium salt]	25 mg
11612	MUP, disodium salt [4-Methylumbelliferyl phosphate, disodium salt]	10 g
11630	PhosLite™ Green	1 mg
11619	pNPP [4-Nitrophenyl phosphate, disodium salt]	25 mg
11013	ReadiUse™ ABTS Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	100 mL
11001	ReadiUse™ ABTS Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	1 L
11012	ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	100 mL
11003	ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	1 L
11010	Signal Guard™ HRP conjugate stabilizer	50 mL
11020	Signal Guard™ HRP reaction stopping solution	0.5 mL
11622	Signal Guard™ phosphatase reaction stopping solution	100 mL
11629	SunRed™ Phosphate	5 mg



Buccutite™ Crosslinking Technology

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