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Introducing CytoCite™ Fluorometer with Cloud-Integration

Palm-sized device with unlimited possiblities

Novel Products & Tools
CytoCite™ Fluorometer with Cloud-integration

Featured Product
Buccuttite™ Antibody Labeling Kits

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Sunnyvale, CA 94085

AAT Bioquest®

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The CytoCite™ Fluorometer is the first cloud-integrated, single channel instrument designed for rapid fluorescence quantitative analysis and assay development. When paired with the appropriate bioassay, the CytoCite™ Fluorometer can measure biomolecules – proteins, nucleic acids, bacteria and other small molecules – with a high degree of sensitivity, accuracy and reproducibility. With its small benchtop footprint, the CytoCite™ Fluorometer can be easily integrated into any laboratory setup, point of care use or field test

Features of the CytoCite™ Fluorometer include:

- A comprehensive library of Portelite™ quantitation kits and fluorimetric reagents
- A blue fluorescence channel: excitation of 457 – 487 nm & emission of 515 – 565 nm
- Greater selectivity and more accurate quantification than absorbance measurements
- Detection of as little as 1 nM of fluorescein (Green channel only)
- Large dynamic range – encompassing five orders of magnitude
- High sensitivity – requires as little as 1 µL of sample volume, perfect for working with precious or limited samples

In addition to providing quantitative measurements that are simple and reliable, the CytoCite™ Fluorometer features an intuitive cloud integrated platform, powered by AAT Bioquest's Device Cloud. This sophisticated device and data management system enables researchers to easily monitor instruments, read samples and organize results when connected to the Device Cloud website.

Through the Device Cloud website, users can securely access all the functions of the software and instrument from the convenience of any popular web browser. This includes data collection, storage and analysis as well as access to AAT Bioquest's Quest Graph™ analytical suite for fast and easy regression modeling, IC_{50} calculations, and more

Features of the Device Cloud platform include:

- End-to-end encryption algorithms to protect valuable data
- Free unlimited storage of your data
- Seamless synchronization of data across multiple platforms, easily accessible from any authenticated device
- Automated daily backups to prevent unexpected data loss
- Convenient data export to Microsoft Excel (*.xls, *.xlsx) as well as comma separated values (*.csv)
- Integration with Quest Graph™ data analysis suite for linear regression modeling, IC50 calculations and more analysis.

Figure 1.1 Schematic illustrating the exterior components of the CytoCite™ BG100 Fluorometer.

CytoCite™ Fluorometer Specifications

The external components of the CytoCite™ Fluorometer, include:

1. **Micro-USB B cable port.** The CytoCite™ Fluorometer must be connected to a computer through the micro-USB port, located on the back of the device, in order to function.
2. **Sample chamber.** This is where the sample containing assay tube must be placed before initiating fluorometric analysis with the instrument.
3. **Power indicator.** LED strip that lights up when the device is successfully paired with the AAT Bioquest Device Cloud software. Please note that simply plugging the CytoCite™ Fluorometer into a computer will not cause the power indicator to light up. It will only light up when actively running through the AAT Bioquest Device Cloud software.
4. **Authenticity sticker.** All authentic CytoCite™ Fluorometer devices will have a hologram sticker as well as a Certificate of Conformity (COC) shipped with the device. Please contact devices@aatbio.com if either is missing.

CytoCite™ Fluorimetric Quantification vs. UV – Absorbance Measurements

Quantification of DNA or protein concentration in a sample is critical for the success of downstream applications such as next-generation sequencing, expression of proteins or qPCR. It can also prevent time consuming troubleshooting and reduce costly errors. For these reasons, scientists prefer a method of quantification that is high in sensitivity and accuracy.

Historically, DNA and protein quantification have been determined using spectrophotometry. This method uses spectrophotometers to measure concentration based on a molecule's absorbance profile at specific wavelength: 260 nm for nucleic acids or 280 nm for proteins. Unfortunately, UV–absorbance suffers from poor selectivity and in certain applications can provide inaccurate readings because spectrophotometers are unable to discern between targets of interest and contaminants that absorb light at the same wavelength.

CytoCite™ fluorimetric quantification easily outperforms absorbance methods in sensitivity and accuracy. Two factors contribute largely to this, the first being instrumentation. The CytoCite™ Fluorometer has the capacity to measure samples with a high degree of linearity covering five orders of magnitude. This enable for more precise and accurate measurements of sample concentrations over a wider range.

Second, CytoCite™ fluorometer utilizes specially designed fluorescent dyes that are highly sensitive and selective to the target of interest. Because these dyes only fluorescence when bound to the target molecule – DNA or protein – fluorescence quantitation is not conflated with noise due to sample contaminants, free nucleotides or buffer elements. Another characteristic of CytoCite™ fluorescent dyes that contribute to assay sensitivity are their high extinction coefficients and fluorescence quantum yields. These properties enable dyes to generate significantly intense fluorescence signals that allow for the detection of molecules at three orders of magnitude lower than what is detectable by traditional absorbance.

What Assays Are Currently Available for the CytoCite™ Fluorometer?

Portelite™ Fluorimetric Quantitation Kits - designed for use with the CytoCite™ Fluorometer - provides a simple and accurate method for quantitating dsDNA, proteins and other small molecules in solution. Portelite™ assays have significant advantages in sensitivity and accuracy over UV absorbance methods for quantitation of DNA, RNA or proteins. Unlike UV absorbance, Portelite™ assays are not affected by the presence of contaminants (e.g. free nucleotides, salts, solvents, etc.), making quantitation much more accurate in complex mixtures such as whole blood or serum.

Each Portelite™ assay is:

- **Ready-to-Use:** no dilution of standards or buffer required
- **Easy-to-Perform:** just dilute the dye in supplied buffer, add the sample and read fluorescence
- **Highly Selective:** assays use target-selective fluorescent dyes that fluoresce only when bound to their respective target, minimizing the effects of contaminants on results
- **Highly Sensitive:** Portelite™ assays are orders of magnitude more sensitive than UV-absorbance measurements.

Portelite™ Fluorimetric High Sensitivity DNA Quantitation Kits

Accurate quantitation of DNA is critical to the success of many downstream applications such as sequencing, PCR amplification and genotyping. The Portelite™ Fluorimetric High Sensitivity DNA Quantitation Kit provides a rapid and sensitive method for quantifying dsDNA over ssDNA, RNA and free nucleotides. Helixyte™ Green used in this assay is a highly-selective dsDNA dye that exhibits a significant fluorescence increase upon dsDNA binding. This assay is linear over four orders of magnitude and is designed to be accurate for sample concentrations from 25 pg/µL to 100 ng/µL.

Table 1.1 Portelite™ DNA Quantitation kit specifications.

Specifications	Portelite™ DNA Assay
Ex/Em	480/520 nm
Target	dsDNA
Initial Sample Concentrations	25 pg/µL to 100 ng/µL
Quantitation Range	0.2 - 100 ng dsDNA
Reagents	Helixyte™ Green
	DNA Assay Buffer
	2 DNA Standards
No. of Assays	100 tests (Cat No. 17660)
	500 tests (Cat No. 17661)

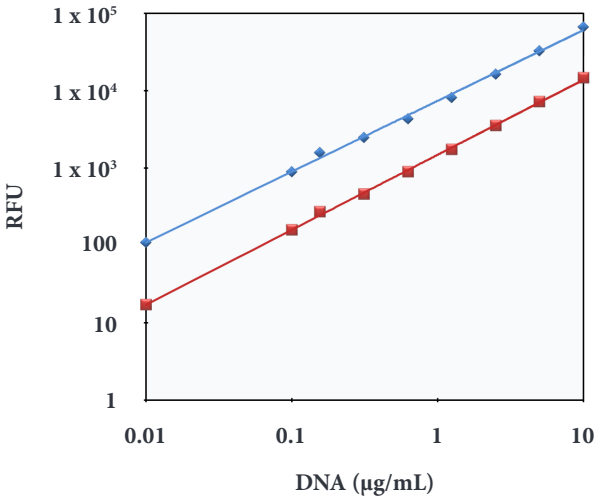

Figure 1.2 DNA standard curves generated using Portelite™ DNA Quantitation Kit comparing the CytoCite™ Fluorometer (Blue) versus the Qubit™ 4 Fluorometer (Red). Fluorescence intensities were quantified using FITC channel, regression models were calculated using log-log best-fit. Detection limit for both devices were comparable at 10 pg/µL (R^2 CytoCite™ = 1, R^2 Qubit™ = 1).

Table 1.2 The following table lists the physical, electrical and optical properties of the CytoCite™ Fluorometer.

Instrument	CytoCite™ Fluorometer
Dimensions	3.54"L x 2.83"W x 1.54"H (9.0 cm x 7.2 cm x 3.9 cm)
Weight	~0.3 lbs (135 g)
Operating Ranges	10 - 30 °C; < 80% relative humidity
Installation Location	Indoor use only
Typical Power Consumption	2.5 VA
Power Requirements	5 VDC, 0.5 A
Computer Interface	Micro-USB B, USB 2.0
Dynamic Range	5 orders of magnitude
Light Source (device dependent)	Blue LED (max ~470 nm)
Excitation Filter (device dependent)	Blue 457 - 487 nm
Emission Filter (device dependent)	Green 515 - 565 nm
Detector	Photodiode
Tube Type	0.2 mL clear, thin-wall PCR tubes
Minimum Assay Volume	150 µL

Table 1.3 Portelite™ Protein Quantitation kit specifications.

Specifications	Portelite™ DNA Assay
Ex/Em	480/520 nm
Target	Protein
Initial Sample Concentrations	12.5 µg/mL to 5 mg/mL
Quantitation Range	0.25 to 5 µg protein
Reagents	Portelite™ Orange
	Sample Dilution Buffer
	3 BSA Standards
No. of Assays	100 tests (Cat No. 11109)
	500 tests (Cat No. 11111)

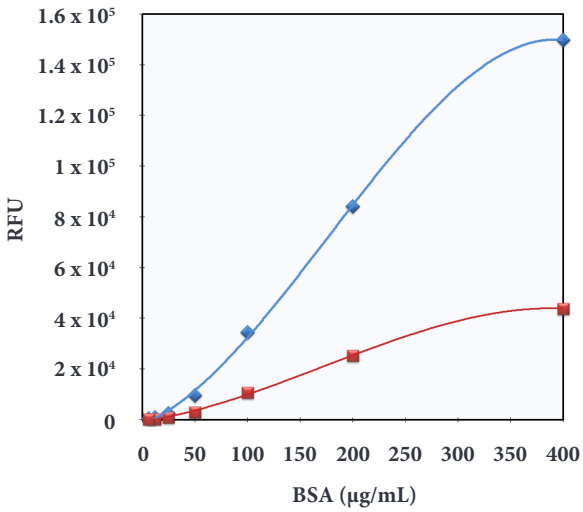

Figure 1.3 BSA standard curves generated using Portelite™ Fluorimetric Protein Quantitation Kit comparing the CytoCite™ Fluorometer (Blue) versus the Qubit™ 4 Fluorometer (Red). Fluorescence intensities were quantified using FITC channel, regression models were calculated using polynomial best-fit. Detection limit for both devices were comparable at 12.5 µg/mL (R^2 CytoCite™ = 1, R^2 Qubit™ = 0.999).

Portelite™ Fluorimetric Protein Quantitation Kits

Protein quantification is an integral part of protein purification, labeling and analysis. Traditional methods, such as the Bradford, Lowry and BCA assays, rely on absorbance-based measurements to quantify proteins. However, these methods are limited in sensitivity range, and require large sample volumes to ensure accuracy.

The Portelite™ Fluorimetric Protein Quantitation Kit is significantly more sensitive than conventional colorimetric protein measurements. The Portelite™ Orange reagent used in this assay is non-fluorescent in aqueous solution, but reacts rapidly with proteins and generates bright fluorescence. This assay can detect as little as 50 ng/mL of protein and is designed to be accurate for sample concentrations from 12.5 µg/mL to 5 mg/mL BSA.

Table 1.4 Product ordering information for CytoCite™ Fluorometer and Portelite™ assay kits.

Cat#	Product Name	Ex (nm)	Em (nm)	Size
CBG100	CytoCite™ BG100 Portable Fluorometer	480	520	Each
CGR100	CytoCite™ GR100 Portable Fluorometer	530	620	Each
CCT100	CytoCite™ Sample Tubes			500 Tubes
17660	Portelite™ Fluorimetric High Sensitivity DNA Quantitation Kit *Optimized for CytoCite™ and Qubit™ Fluorometers*	501	520	100 Tests
17661	Portelite™ Fluorimetric High Sensitivity DNA Quantitation Kit *Optimized for CytoCite™ and Qubit™ Fluorometers*	501	520	500 Tests
11109	Portelite™ Fluorimetric Protein Quantitation Kit *Optimized for CytoCite™ and Qubit™ Fluorometers*	485	590	100 Tests
11111	Portelite™ Fluorimetric Protein Quantitation Kit *Optimized for CytoCite™ and Qubit™ Fluorometers*	485	590	500 Tests

Buccutite™ Conjugation Technology

Prepare High-Performance PE, APC & Tandem Conjugates for Flow Cytometry

The use of phycobiliprotein labeled antibody conjugates has important applications in flow cytometry. Their usefulness is attributed to their individual brightness as well as their ability to create spectral separation when used as tandem dyes for multicolor analysis. A common approach for making PE, APC or tandem antibody conjugates is to use a succinimidyl 4-(N-maleimidomethyl) cyclohexane1-carboxylate (SMCC) crosslinking method.

This method uses a heterobifunctional crosslinker SMCC. SMCC crosslinkers contain N-hydroxysuccinimide (NHS) ester and maleimide groups, which allow covalent conjugation of amine (-NH2) and sulfhydryl (-SH) containing molecules. NHS esters react with primary amines at pH 7-9, while malemides react with sulfhydryl groups at pH 6.5-7.5. Because the rate of NHS esters hydrolytic degradation increases with pH, the NHS ester reaction usually occurs before or simultaneous with the maleimide reaction. For users not adept to conjugation chemistry, this may be challenging.

Although SMCC is a well-established method, it has its disadvantages. One of the major drawbacks includes poor conjugation efficiency, with a good yield at approximately 30% recovery. Secondly, SMCC requires the addition of dithiothreitol (DTT), which may significantly impact antibody immunoreactivity. Lastly, because the efficiency of SMCC-based reactions is typically low, very high input (i.e. PE and antibody) is required for successful conjugation.

Buccutite™ Technology – Simplified Workflow

An alternative to SMCC conjugation is AAT Bioquest's novel Buccutite™ technology. Buccutite™ conjugation is a quick and simple technique for labeling PE, APC or tandem dyes to antibodies. It utilizes two separate linkers, Buccutite™ MTA and Buccutite™ FOL. These linkers are independently labeled to the phycobiliprotein and antibody of interest, and when mixed will bind strongly together to produce a bioconjugate.

To improve workflow and reduce hands-on time, Buccutite™ phycobiliprotein labeling kits are supplied with the fluorescent protein pre-activated with the Buccutite™ FOL linker. After antibody activation with Buccutite™ MTA, linker, both components are mixed and antibody conjugates are produced. The following diagram is an overview of the protocol for Buccutite™ Rapid PE Antibody Labeling Kit (Cat# 1310).

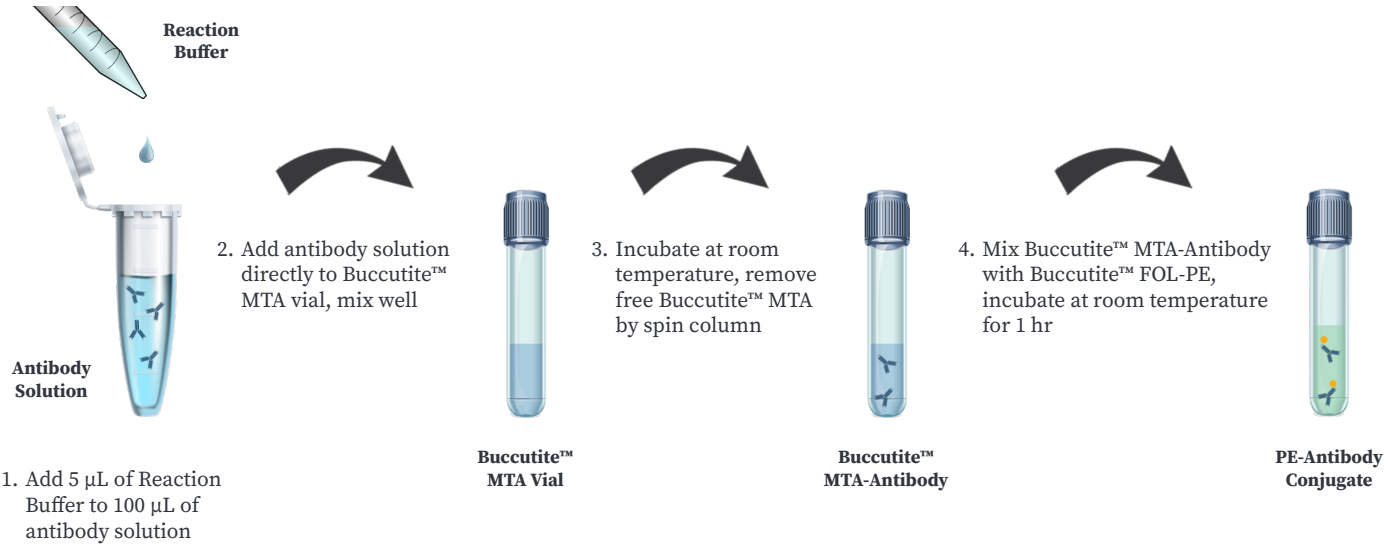

Figure 2.1 Diagram illustrating a general procedure for Buccutite™ Rapid PE Antibody Labeling Kit (Cat No. 1310).

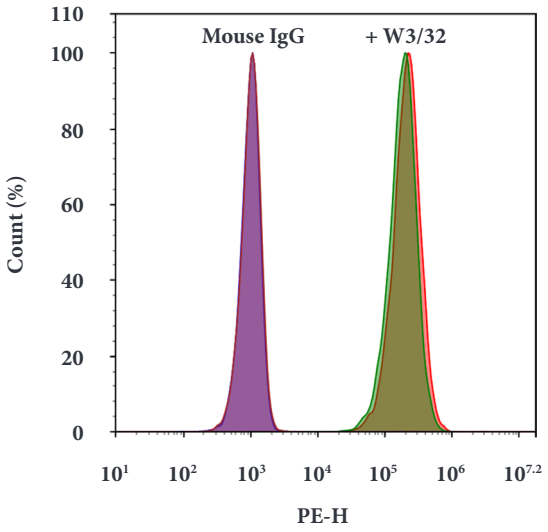

Figure 2.2 Performance of GXM IgG-PE conjugates prepared with unstained control cells (blue), Mouse IgG unconjugated control, Buccutite™ conjugates (Green) and SMCC conjugates (Red). HL-60 cells were stained with or without w6/32 antibody (1ug/ml) for 30min, and followed by GXM IgG-PE (5ug/ml) for 30min. The fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the PE channel.

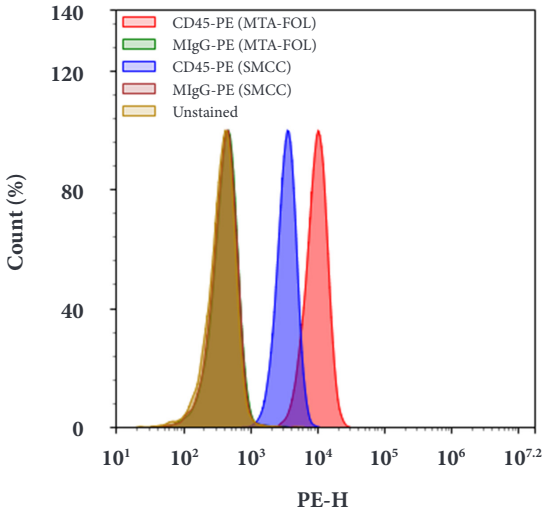

Figure 2.3 Flow cytometry analysis of Jurkat cells stained with CD45-PE with different method (Buccutite™: Red; SMCC: Blue). The CD45-PE conjugate was not purified for both reaction and fluorescence signal was monitored using ACEA NovoCyte flow cytometer in the PE channel.

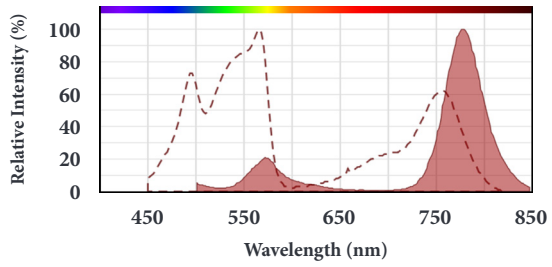

Figure 2.4 Excitation and emission spectra of PE-Cy7 (Cat# 2616).

Buccutite™ vs. SMCC Conjugation

Buccutite™ offers several advantages over SMCC-based conjugation. First, Buccutite™ conjugation efficiency is relatively high, with final recovery more than double (>60%) that of SMCC conjugation. Second, the Buccutite™ reaction is efficient, conjugation can occur at low concentrations of reactants requiring a minimal sample concentration ≥0.5 mg/mL. Lastly, Buccutite™ conjugates are highly stable and can be stored at 4 °C for at least 12 months (see below for data).

In the following comparison, mouse monoclonal antibody conjugates (GXM) were prepared using Buccutite™ and SMCC conjugation. The conjugates were used to perform cell stains for flow cytometry (Figure 1). Results illustrate similarities in the positive stain and stain indexes of both conjugates, highlighting Buccutite™ as a successful alternative to SMCC.

Figure 2 illustrates the high labeling efficiency of the Buccutite™ conjugation reaction comparable to the SMCC method. More importantly, we discovered that, even without the column purification step Buccutite™ conjugation kits produced fluorescent conjugates far superior to those of unpurified SMCC conjugates. Unpurified SMCC conjugates saw a decrease in stain index by 50%.

Buccutite™ Conjugates vs. Commercial Conjugates

PE-Cy7 (Cat# 2616) is a popular tandem dye commonly used in combination with FITC (Cat# 135), PE (Cat# 2558) and other tandem dyes for multi-parameter flow cytometry. PE-Cy7 consists of a PE phycobiliprotein labeled with the cyanine dye, Cy7 (Cat# 161). When excited by the 488 laser, PE functions as a donor transferring energy to Cy7 via Förster resonance energy transfer (FRET) to emit fluorescence at 780 nm. This difference of 292 nm between the maximal excitation and maximal emission is referred to as a Stoke's shift. Large Stoke's shift of tandem dyes, such as PE-Cy7, is extremely valuable when designing multicolor flow cytometry panels because it provides considerable spectral separation and minimizes crosstalk.

In the following study, PE-Cy7-streptavidin conjugates prepared using Buccutite™ Rapid PE-Cy7 Tandem Labeling Kit (Cat# 1317) were compared with PE-Cy7-strepdavidin conjugates purchased from BioLegend. The performance of both conjugates was tested in HL-60 cells. Cells were stained with 1 µg/mL CD45-Biotin or mouse IgG biotin (control) and then stained with PE-Cy7-streptavidin conjugates. Results illustrate similar performance in staining,

indicating Buccutite™ conjugation technology as a valuable alternative for conjugate production.

Stability of Buccutite™ Reagents

A key advantage of Buccutite™ conjugation kits is the stability of its components. Both the Buccutite™ MTA linker and the pre-activated PE, APC or tandem dye can be lyophilized into powder form and stored at 4°C for at least 12 months without compromising product integrity. Stability was tested using CD45 antibody conjugates labeled with Buccutite™ FOL-Activated PE (Component A in Cat# 1310) and Buccutite™ FOL-Activated APC (Component A in Cat# 1311) stored at 4°C for 12 months. CD45 antibodies were pre-activated with Buccutite™ MTA (Component B in Cat# 1310 and Cat# 1311) and reacted with reconstituted Buccutite™ FOL-Activated PE and Buccutite™ FOL-Activated APC. For control, CD45 antibodies were also labeled with freshly prepared Buccutite™ FOL-Activated PE and Buccutite™ FOL-Activated APC. Jurkat cells were stained with the conjugates without further purification, and performance is shown in Figure 5. The brightness and signal-to-background ratio showed little variation between fresh Buccutite™ FOL-Activated PE/APC and Buccutite™ FOL-Activated PE/APC stored at 4°C for 12 months

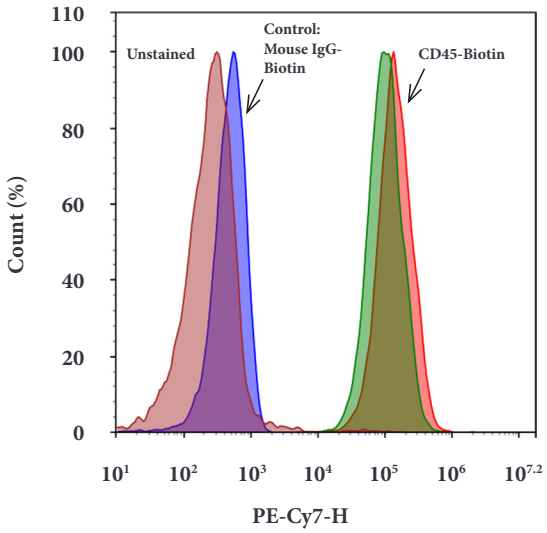

Figure 2.5 Performance of SA-PE/Cy7 conjugate prepared with Buccutite™ method were compared with SA-PE/Cy7 (BioLegend). HL-60 cells were stained with or without CD45 antibody (1ug/ml) for 30min, and followed by SA -PE/Cy7 (5ug/ml) for 30min. (Red peak: SA-PE/Cy7 (Buccutite™), Green peak: SA-PE/Cy7 (BioLegend).

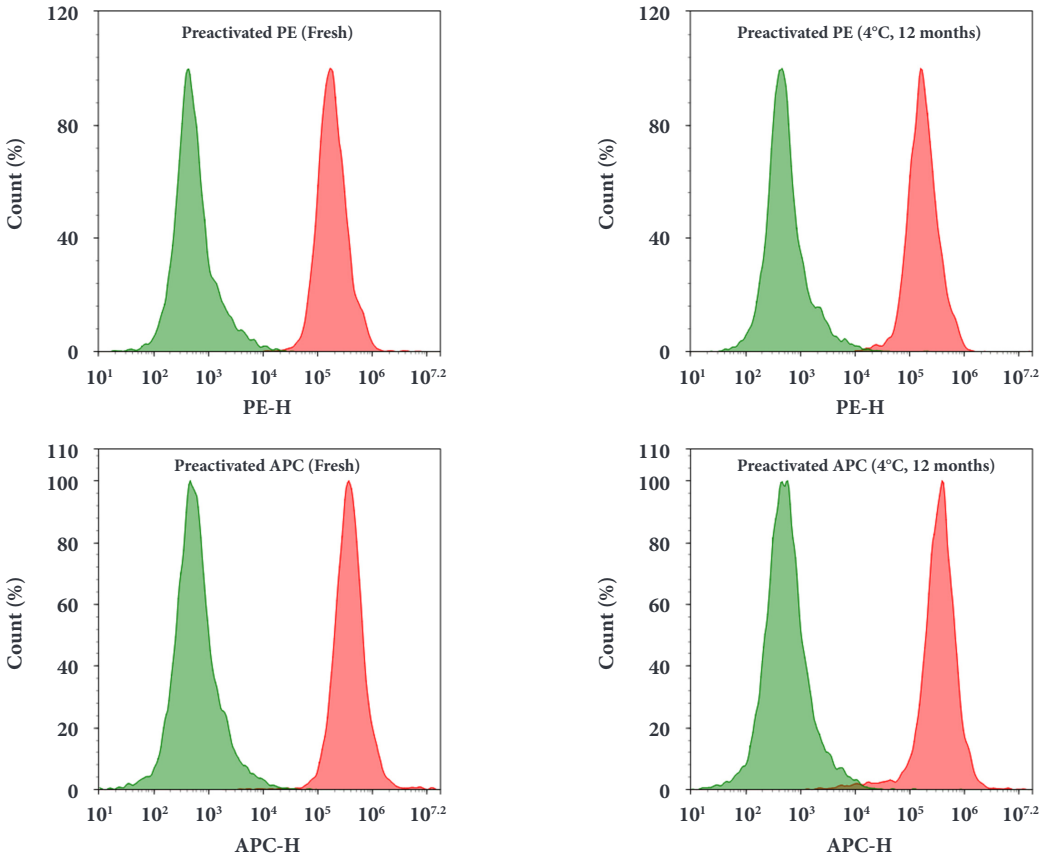

Figure 2.6 Flow cytometry analysis of Jurkat cells stained with CD45-PE or CD45-APC prepared with Buccutite™ Kit (Cat#1310, & cat#1311). Conjugates were prepared with fresh pre-activated PE (or APC) in liquid form and lyophilized form (4oC one year old). The performance on Jurkat cell was compared using ACEA NovoCyte flow cytometer. (Green: Mouse IgG conjugate control, Red: CD45-Conjugate).

Available Buccutite™ Labeling Kits

Buccutite™ Rapid Antibody Conjugation Kits are available for labeling antibodies and proteins with PE, APC or Tandem dyes. The phycobiliprotein or tandem dye in each kit is pre-activated with Buccutite™ FOL, and can be directly conjugated to proteins of interest activated with Buccutite™ MTA. The Buccutite™ FOL -activated dye readily reacts with Buccutite™ MTA-containing molecules under extremely mild neutral conditions without any catalyst required. Compared to commonly used SMCC.

Buccutite™ Rapid PE and PE-Tandem Antibody Labeling Kits

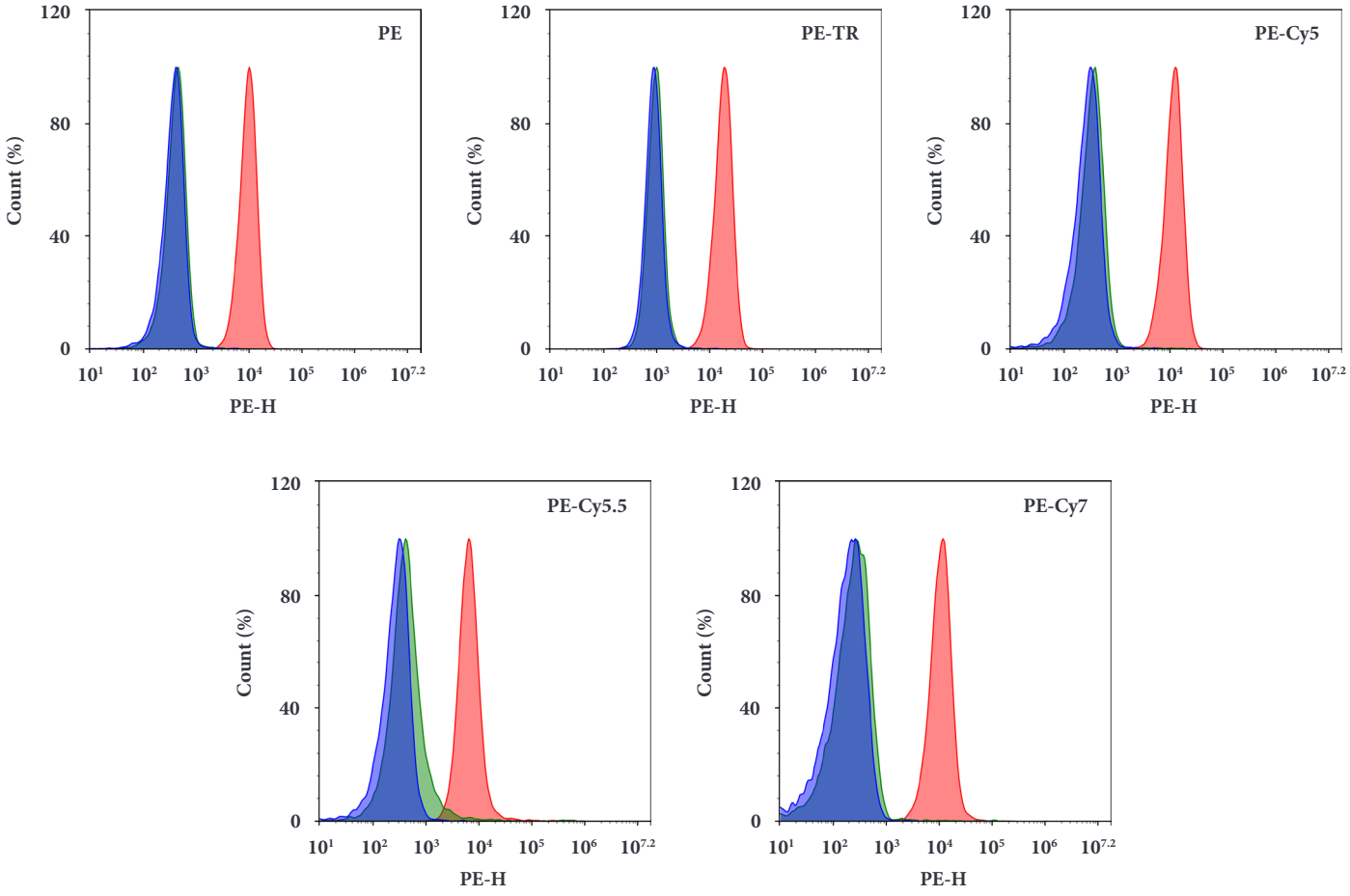

Figure 2.7 Flow cytometry analysis of Jurkat cells stained with CD45-PE or PE Tandems prepared with Buccutite™ Kit (Cat#1310, 1316, 1317, 1318, and 1322). Jurkat cells were stained with PE conjugate and analyzed with ACEA NovoCyte flow cytometer. (Blue: Unstained cells, Green: Mouse IgG conjugate control, Red: CD45-Conjugate).

Table 2.1 Buccutite™ Rapid PE Antibody Labeling Kit (2 Conjugations/Kit, Each Labeling is for 100 µg Antibody).

Cat#	Product Name	Fluorophore
1310	Buccutite™ Rapid PE Antibody Labeling Kit	PE
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit	PE-Cy5.5
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit	PE-Cy7
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit	PE-Texas Red
1322	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit	PE-Cy5

Buccutite™ Rapid APC and APC-Tandem Antibody Labeling Kits

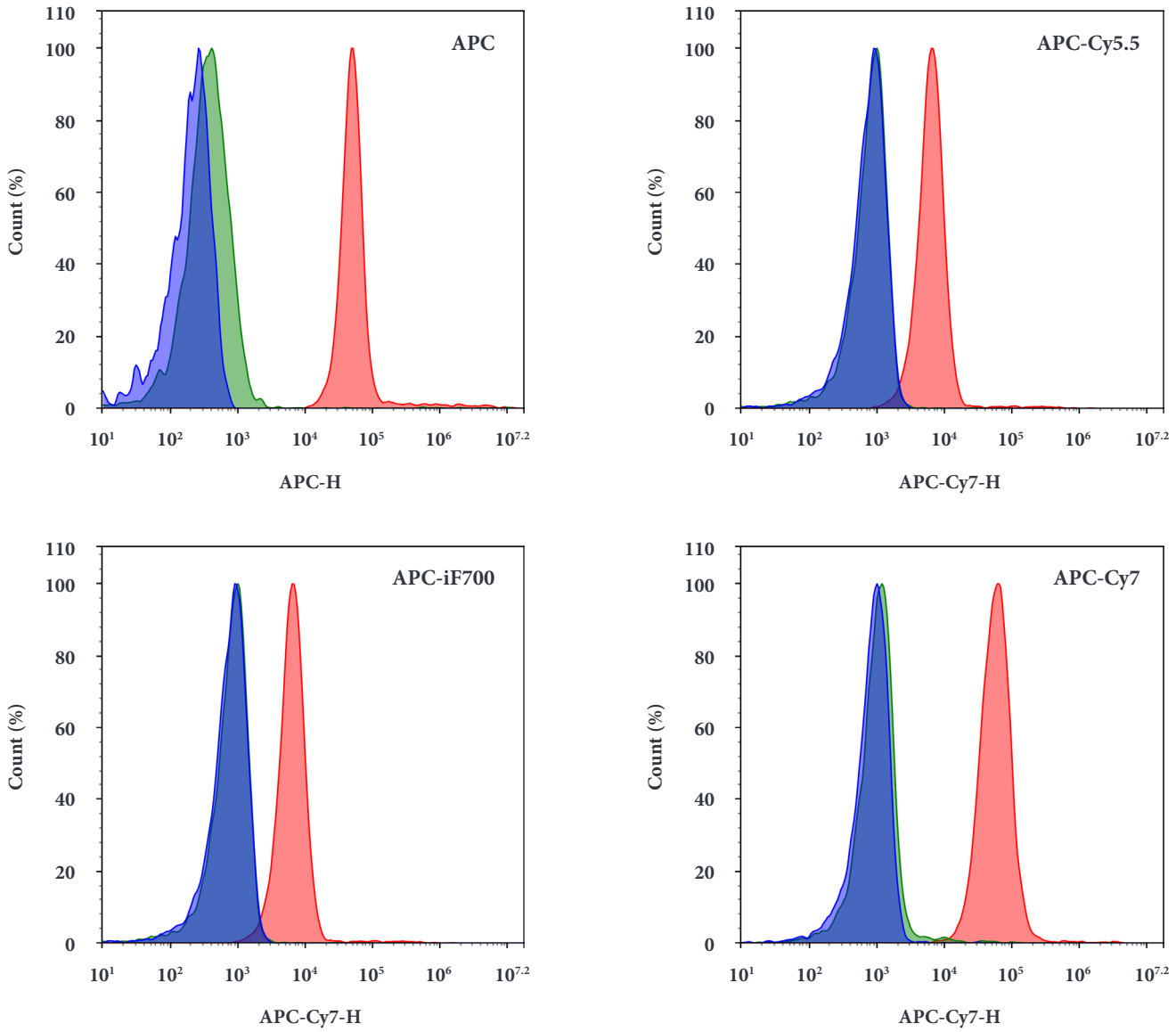

Figure 2.8 Flow cytometry analysis of Jurkat cells stained with CD45-APC or APC Tandems prepared with Buccutite™ Kit (Cat#1311, 1319, 1320, and 1321). Jurkat cells were stained with APC conjugate and analyzed with ACEA NovoCyte flow cytometer. (Blue: Unstained cells, Green: Mouse IgG conjugate control, Red: CD45-Conjugate).

Table 2.2 Buccutite™ Rapid APC Antibody Labeling Kit (2 Conjugations/Kit, Each Labeling is for 100 µg Antibody).

Cat#	Product Name	Fluorophore
1311	Buccutite™ Rapid APC Antibody Labeling Kit	APC
1319	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit	APC-iFluor™ 700
1320	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit	APC-Cy5.5
1321	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit	APC-Cy7

Conclusion

- Pre-activated phycobiliprotein and the tandems are stable for at least 12 months with the similar activity in Buccutite™ reactions, without significant changes in performance.
- Buccutite™ Ab conjugation kit is suitable to make Ab conjugate with good performance.
- The conjugates prepared with Buccutite™ technology are compatible with all other commercial conjugates, and also compatible with each other in cell surface staining for analysis and sorting.

Product Ordering Information

Table 2.3 Product ordering information for Buccutite™ PE, APC and tandem dye antibody labeling kits.

Cat#	Product Name	Ex (nm)	Em (nm)	Size
1310	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565	575	2 Labelings
1311	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651	662	2 Labelings
1312	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	565	575	2 Labelings
1313	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	651	662	2 Labelings
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565	700	2 Labelings
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565	780	2 Labelings
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651	600	2 Labelings
1319	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651	713	2 Labelings
1320	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	651	700	2 Labelings
1321	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*	565	780	2 Labelings
1322	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	565	674	2 Labelings
1325	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	482	677	2 Labelings
1340	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	674	2 Labelings
1341	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	700	2 Labelings
1342	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	780	2 Labelings
1343	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	565	600	2 Labelings
1347	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	651	713	2 Labelings
1350	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	651	700	2 Labelings
1351	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	651	780	2 Labelings
1353	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 25 µg Antibody Per Reaction*	482	677	2 Labelings

ReadiView™ Biotin Reagents

All-In-One Biotinylation & Quantification of Biotin Labeling

The biotin-streptavidin complex is one of the most popular tagging systems for the conjugation of biomolecules, such as proteins, lipids and nucleic acids, as well as that of synthetic molecules, such as fluorescent labels. It has found strong success in the area of sample preparation as a core part of many affinity purification systems, and plays a critical role in many detection systems for instruments such as microscopy and flow cytometry.

The widespread adoption of the biotin-streptavidin conjugation system is primarily due to the extraordinarily high binding affinity of streptavidin for biotin. This complex is one of the strongest known non-covalent interactions between a protein and ligand, and permits binding of biotinylated molecules to streptavidin conjugates in heterogeneous mixture. Bond formation is rapid, and able to withstand extremes in pH, temperature, organic solvents, and denaturing agents.

A key benefit to the biotin-streptavidin complex is its ability to significantly improve the detection sensitivity of any assay. This in large part is due to the tetrameric conformation of streptavidin, which enables streptavidin to bind four biotins per molecule with high affinity and selectivity. This multiplicity allows for amplification of weak signals, allowing for improved experimental results when testing for medium- to low-abundance targets in mammalian cells or tissues (Figure 1).

Biotinylation

Biotinylated proteins are routinely used in conjunction with streptavidin conjugates. Together, they form a sophisticated detection platform that is readily adaptable to a broad range of applications. A large factor that contributes to the versatility of the biotin-streptavidin system is the ability to conjugate streptavidin to a variety of reporter tags. For example, enzyme conjugates of streptavidin, such as HRP-streptavidin (Cat# 16920) or AP-streptavidin (Cat# 16921), are commonly used in immunoblotting, ELISA and in situ hybridization imaging applications. While fluorescently labeled streptavidin, such as iFluor™-streptavidin, are widely used in cell surface labeling, fluorescence activated cell sorting (FACs) and other fluorescence imaging applications.

Biotin is a 244 dalton vitamin that is present in trace amounts in all living cells. Besides having a strong affinity for streptavidin, biotin exhibits two characteristics that make it ideal for bioconjugate development. First, is biotin's relatively small size. This minimizes any significant hindrance with a protein's

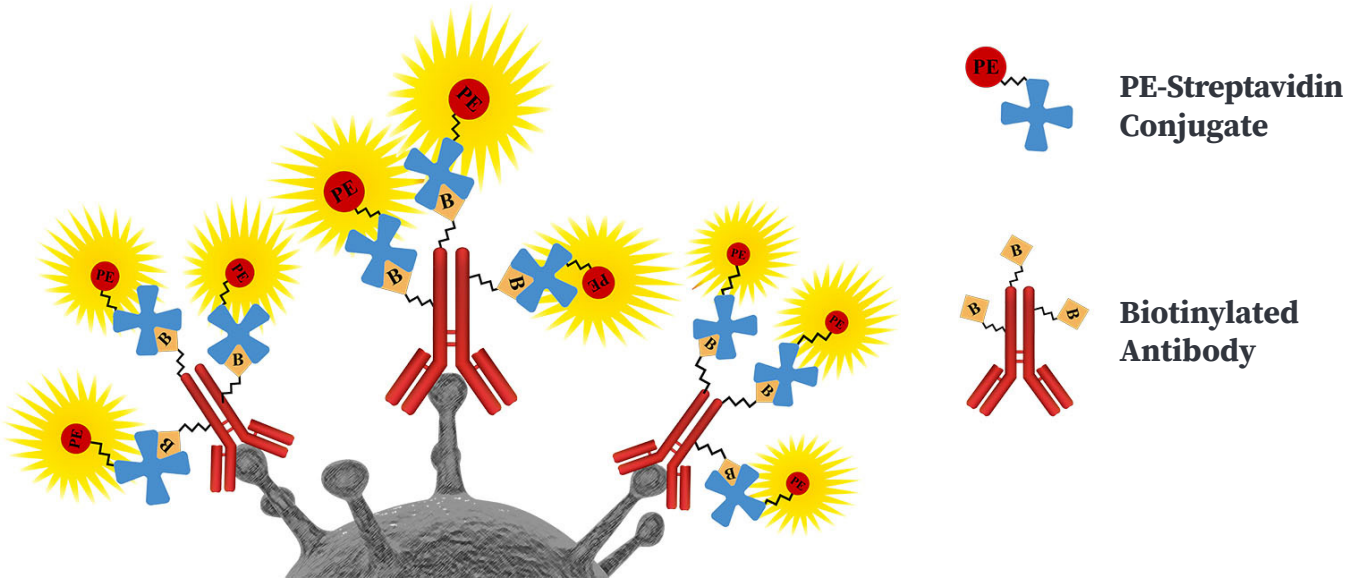

Figure 3.1 Shematic of signal amplification by biotin-streptavidin formation.

biological reactivity, and permits the biotinylation – labeling – of multiple biotin tags to a single macromolecule for maximum detection by streptavidin and its conjugates.

The second characteristic is the valeric acid side chain of the biotin molecule. This chain can be derivatized to incorporate various reactive groups used to biotinylate proteins without altering its binding affinity for streptavidin. This feature is critical as it allows for the development of various biotinylated reagents. These include, amine-reactive biotin succinimidyl ester and thiol-reactive biotin maleimide, as well as, ReadiView™ reactive biotins and ReadiLink™ Protein Biotinylation Kit (Cat #5521) which enable researchers to chemically label proteins, nucleic acids and other molecules in order make custom assay probes.

Biotinylation Reagents

Biotinylation can be performed enzymatically or chemically. Of the two methods, chemical biotinylation is more commonly utilized because it offers greater flexibility and can be performed in vitro and in vivo. Biotinylation reagents are typically comprised of three components, which include the biotin tag, a spacer arm and a reactive group. As previously mentioned, the valeric acid side chain of biotin is modified with reactive groups, this dictates target reactivity. Common protein targets for chemical modification include primary amines (-NH2), thiols (-SH), carboxyls (-COOH) and carbonyls (-CHO). Of the four, primary amines are the most targeted functional group because of their abundance and accessibility. Succinimidyl esters (SE) form stable amide bonds with primary amines and can readily be incorporated on biotin.

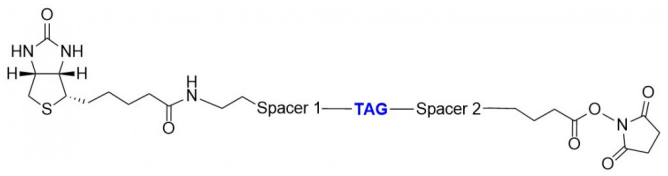

Figure 3.2 ReadiView™ biotin conjugated with specially designed color tag (CT) for easy determination of biotinylation degree.

The distance between the reactive group and biotin tag can be adjusted using spacer arms of various lengths, which can increase the availability of biotin for streptavidin binding, reduce steric hindrance and increase reagent solubility. Spacer arm length is commonly regulated by hydrocarbons, PEG or disulfide bonds that allow for biotin cleavage. When determining the appropriate biotinylation reagent to use for a specific application and protein of interest carefully consider: (1) solubility, (2) spacer arm length, (3) functional group, and (4) degree of biotinylation.

Step 4, quantification of the degree of biotinylation, is useful in order achieve and maintain a high degree of consistency of reagents used in research and diagnostic setting. Quantification of the degree of the biotinylation reaction can assist in optimizing a particular biotin-streptavidin system and ensure reproducibility in the biotinylation processes. However, it is challenging to quantitate biotin directly because its intrinsic absorption is difficult to distinguish from that of proteins and nucleic acids. Therefore, in order to quantitate biotinylation a separate method is required; this increases cost and hands-on-time.

The 4'-hydroxyazobenzene-2-carboxylic acid or HABA assay (Appendix A) is one such technique. Besides increasing cost and time, the HABA assay and similar streptavidin binding assays for measuring biotinylation suffer from numerous shortcomings, including poor sensitivity, requiring and consuming large sample volumes (up to 75 µg of labeled protein) and requiring external streptavidin-calibration curves.

Quantification of Biotinylation using ReadiView™ Biotin

To address these concerns, AAT Bioquest has designed ReadiView™ biotin (Figure 2). This family of reactive biotins includes five novel biotinylation reagents in various reactive

Table 3.1 ReadiView™ Biotin target reactivity.

Cat#	Product Name	Functional Group Target
3059	ReadiView™ Biotin Succinimidyl Ester	Primary Amines (-NH_2)
3058	ReadiView™ Biotin Maleimide	Thiols or Sulfhydryls (-SH)
3050	ReadiView™ Biotin Acid	Amines (-NH_2)
3055	ReadiView™ Biotin Hydrazide	Carbonyls (-CHO)
3053	ReadiView™ Biotin Amine	Carbonyls (-CHO)

formats: succinimidyl ester, maleimide, acid, hydrazide, and amine. The ReadiView™ biotin series has been optimized to streamline the biotinylation and quantification processes. Each ReadiView™ biotin reagent contains a specially designed Color Tag (CT) optimally positioned between two spacer arms. These spacer arms reduce steric hindrance and improve solubility, while the CT tag makes the degree of biotinylation readily quantifiable by calculating the absorption ratio of 280 nm/385nm. Additionally, the CT tag has minimal effect on the biotin binding affinity and minimal quenching effect on fluorophores used to label streptavidin.

Sample Protocol - ReadiView™ Biotin SE

The following is an overview of the protocol for ReadiView™ biotinylation and quantification of the biotinylation using ReadiView™ Biotin succinimidyl ester (SE):

Running the conjugation reaction:

1. Add the protein working solution into the vial of ReadiView™ Biotin SE stock solution (2 µL/vial), and mix them well by repeatedly pipetting for a few times or vortex the vial for 2-5 minutes.
2. Keep the conjugation reaction mixture at room temperature for 30 – 60 minutes.
   Note: The conjugation reaction mixture can be rotated or shaken for longer time if desired.

Preparation of the spin column for sample purification:

1. Invert the provided spin column several times to re-suspend the settled gel and remove any bubbles.
2. Snap off the tip and place the column in a washing tube (2 mL). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube. However, centrifuge immediately if the column is placed into a 12 x 75 mm test tube.
3. Centrifuge for 1 minute in a swinging bucket centrifuge at 1,000 x g to remove the packing buffer. Discard the buffer.
4. Apply 1-2 mL 1X PBS (pH 7.2-7.4) to the column. After each application of PBS, let the buffer drain out by gravity, or centrifuge the column for 2 minutes to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.
5. Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g (see Centrifugation Notes section) to remove the packing buffer. Discard the buffer.

Purification of the conjugates:

1. Place the column in a clean Collecting Tube (1.5 mL). Carefully load the sample (20-100 µL, from Step conjugation reaction) directly to the center of the column.
2. After loading the sample, add 1X PBS (pH 7.2-7.4) to make the total volume of 110 µL. Centrifuge the column for 5-6 minutes at 1,000 x g, and collect the solution that contains the desired biotin-labelled protein.

Quantification of the biotinylation process:

1. Measure the absorption at 280 and 389 nm and calculate the number of biotin moieties displaced on your target protein.

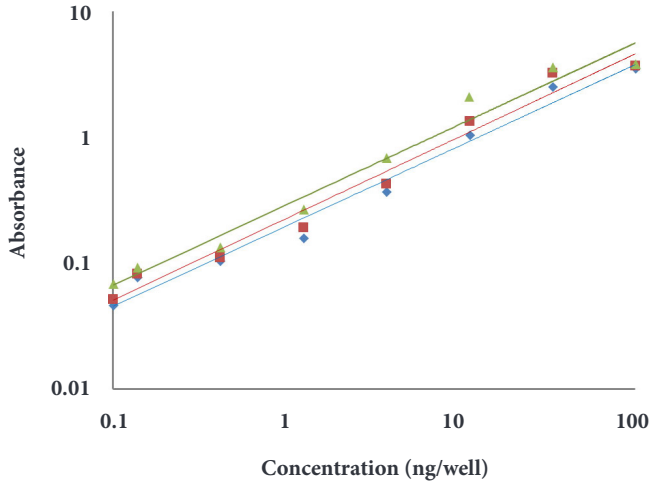

Figure 3.3 Sensitivity assay performed using an ELISA on a known concentration of Mouse IgG antibody by applying Biotinylated Goat anti-mouse antibody, modified by ReadiView™ biotin succinimidyl ester (Cat# 3010), ReadiLink™ Protein Biotinylation Kit (Cat# 5521) or bought from vendor A, followed by Streptavidin-HRP (Cat# 16920). The colorimetric signal was generated using ReadiUse™ TMB Substrate Solution (Cat# 11003).

Appendix A: Common Methods For Measuring Biotinylation

HABA

The most common method to measure the degree of biotinylation of a sample is using 4'-hydroxyazobenzene-2-carboxylic acid (HABA) dye, which non-covalently binds to avidin in the absence of biotin. When bound to avidin, HABA exhibits an absorbance at 500 nm (A500), which is proportional to the amount of bound HABA. When a biotinylated sample is mixed with the HABA–avidin complex solution, the biotin displaces HABA for binding to avidin because the association constant of the avidin–biotin interaction is much greater than that for HABA–avidin (6 x 106 M-1). Because the absorbance of HABA is proportional to its binding to avidin, the amount of biotin present in the solution can be calculated based on the reduction in the A500 signal. The traditional colorimetric HABA assay requires lot of samples approx. (20 µL) and has sensitivity around 2 to 16 µM.

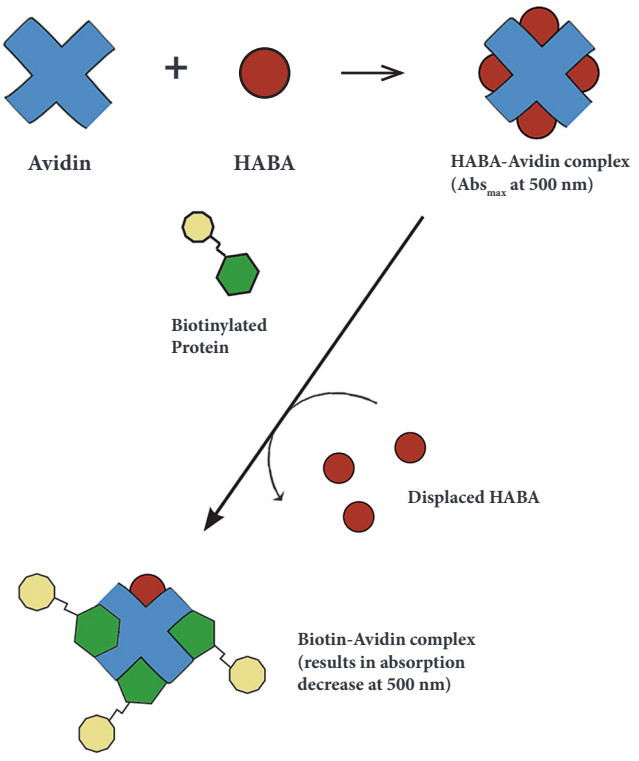

Figure 3.4 HABA assay principle for quantifying degree of biotinylation.

ELISA

Another method for the quantification of biotinylation is enzyme linked immunosorbent assay (ELISA). Biotinylated proteins are immobilized onto polystyrene microtiter plates, The free proteins are washed away and non-specific binding of proteins was blocked using the blocking buffer. The plate then will be incubated with streptavidin alkaline-phosphatase. The amount of streptavidin-enzyme conjugate retained in the wells is detected by yellow color produced by adding p-nitrophenyl phosphate which is measured at 405 nm wavelength. The amount of signal generated is directly proportional to the amount of biotinylated protein absorbed to the microtiter plate well surface.

SDS-PAGE

The presence of biotinylated and non-biotinylated proteins in a sample can also be estimated by binding of the sample to the streptavidin coated magnetic beads by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The test sample is incubated with the SA-coated magnetic beads for 30 minutes and the amount of non-bound can be compared to input. Now, this technique is based on the assumption that SA-coated beads are in excess to the amount of biotin attached to the protein. Also, there are chance of non-specific binding of the non-biotinylated protein to the beads.

References

1. Hofmann K et al. (1982) Avidin binding of carboxyl-substituted biotin and analogues. Biochemistry 21:978–984
2. Hirsch J et al. (2002) Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. Analytical Biochemistry 308:343–357
3. Sugawara K et al. (2005) Voltammetric homogeneous binding assay of biotin without a separation step using iminobiotin labeled with an electroactive compound. Anal Sci 21:897–900
4. Hermanson GT (2008) Bioconjugate techniques, 2nd Edition. San Diego (CA): Academic Press
5. Barat B, Wu AM (2007) Metabolic biotinylation of recombinant antibody by biotin ligase retained in the endoplasmic reticulum. Biomol Eng 24:283–91

Product Ordering Information

Table 3.2 Product ordering information for biotin and streptavidin labeling tools, kits and conjugates.

Cat#	Product Name	Ex (nm)	Em (nm)	Size
3001	Biotin *CAS 58-85-5*			1 g
3002	Biotin, succinimidyl ester *CAS 35013-72-0*			100 mg
3003	Biotin ethylenediamine *CAS 216299-38-6*			10 mg
3004	Biotin cadaverine			25 mg
3005	Biotin C2 maleimide			25 mg
3006	Biotin-4-fluorescein *CAS 1032732-74-3*	492	518	5 mg
3007	Biotin hydrazide *CAS 66640-86-6*			25 mg
3009	Biotin-X NTA [Biotin-X nitrilotriacetic acid, potassium salt] *CAS 856661-92-2*			1 mg
3010	Biotin-X, succinimidyl ester *CAS 72040-63-2*			25 mg
3014	Biotin PEG2 amine *CAS 138529-46-1*			5 mg
3015	Biotin PEG2 maleimide *CAS 305372-39-8*			5 mg
3016	Biotin PEG2 succinimidyl ester			25 mg
3017	Fluorescein biotin	492	515	5 mg
3019	Biotin-PEG3-azide *CAS 945633-30-7*			5 mg
3020	Biotin Azide			5 mg
3021	Biotin Alkyne *CAS 1006592-45-5*			5 mg
3022	Biotin PEG4 succinimidyl ester			25 mg
3024	Biotin PEG3 amine			25 mg
3050	ReadiView™ biotin acid			25 mg
3053	ReadiView™ biotin amine			5 mg
3055	ReadiView™ biotin hydrazide			5 mg
3058	ReadiView™ biotin maleimide			5 mg
3059	ReadiView™ biotin succinimidyl ester			5 mg
3100	Cy5 biotin conjugate	650	669	5 mg
5520	ReadiLink™ Protein Biotinylation Kit			2 Labelings
5521	ReadiLink™ Protein Biotinylation Kit *Powered by ReadiView™ Biotin Visionization Technology*			1 kit
5522	Amplite™ Colorimetric Biotin Quantitation Kit	500		200 Tests
16729	Biotinylated goat anti-mouse IgG (H+L)			1 mg
16794	Biotinylated goat anti-rabbit IgG (H+L)			1 mg
16890	AF350-streptavidin conjugate [Streptavidin, Alexa Fluor™ 350 Conjugate]	346	445	1 mg
16891	AF488-streptavidin conjugate [Streptavidin, Alexa Fluor™ 488 Conjugate]	494	517	1 mg
16892	AF594-streptavidin conjugate [Streptavidin, Alexa Fluor™ 594 Conjugate]	590	617	1 mg
16900	RPE-streptavidin conjugate	565	575	100 ug
16901	RPE-streptavidin conjugate	565	575	1 mg
16902	APC-streptavidin conjugate	651	662	100 ug
16905	PerCP-streptavidin conjugate	482	667	100 ug
16906	RPE-iFluor™ 647-streptavidin conjugate	565	674	100 ug
16907	RPE-iFluor™ 750-streptavidin conjugate	565	779	100 ug
16908	APC-iFluor™ 750-streptavidin conjugate	651	779	100 ug

Table 3.2 Cont. Product ordering information for biotin and streptavidin labeling tools, kits and conjugates.

Cat#	Product Name	Ex (nm)	Em (nm)	Size
16910	FITC-streptavidin conjugate	492	515	1 mg
16911	Texas Red®-streptavidin conjugate	582	602	1 mg
16912	Cy3®-streptavidin conjugate	555	565	1 mg
16913	Cy5®-streptavidin conjugate	649	665	1 mg
16914	Cy7®-streptavidin conjugate	749	776	1 mg
16916	RPE-Cy7-streptavidin conjugate	565	779	100 ug
16917	RPE-Cy7-streptavidin conjugate	565	779	1 mg
16920	HRP-streptavidin conjugate			1 mg
16921	AP-streptavidin conjugate [Streptavidin-alkaline phosphatase conjugate]			1 mg
16925	trFluor™ Eu-streptavidin conjugate	346	617	100 ug
16926	trFluor™ Tb-streptavidin conjugate	330	544	100 ug
16930	mFluor™ Violet 450-streptavidin conjugate	403	454	100 ug
16931	mFluor™ Violet 510-streptavidin conjugate	414	508	100 ug
16932	mFluor™ Violet 540-streptavidin conjugate	405	537	100 ug
16935	mFluor™ Blue 570-streptavidin conjugate	553	570	100 ug
16938	mFluor™ Green 620-streptavidin conjugate	523	617	100 ug
16942	mFluor™ Yellow 630-streptavidin conjugate	611	630	100 ug
16946	mFluor™ Red 700-streptavidin conjugate	657	700	100 ug
16948	mFluor™ Red 780-streptavidin conjugate	629	780	100 ug
16980	iFluor™ 350-streptavidin conjugate	345	442	1 mg
16982	iFluor™ 405-streptavidin conjugate	401	420	1 mg
16985	iFluor™ 488-streptavidin conjugate	491	514	1 mg
16986	iFluor™ 514-streptavidin conjugate	511	527	1 mg
16987	iFluor™ 532-streptavidin conjugate	536	560	1 mg
16989	iFluor™ 555-streptavidin conjugate	552	567	1 mg
16992	iFluor™ 594-streptavidin conjugate	592	614	1 mg
16995	iFluor™ 633-streptavidin conjugate	638	655	1 mg
16996	iFluor™ 647-streptavidin conjugate	654	674	1 mg
16997	iFluor™ 680-streptavidin conjugate	682	701	1 mg
16998	iFluor™ 700-streptavidin conjugate	693	713	1 mg
16999	iFluor™ 750-streptavidin conjugate	753	779	1 mg
17016	Biotin-11-dUTP *1 mM in Tris Buffer (pH 7.5)* *CAS 86303-25-5*			25 nmoles
17017	Biotin-16-dUTP *1 mM in Tris Buffer (pH 7.5)* *CAS 136632-31-0*			25 nmoles
17018	Biotin-20-dUTP *1 mM in Tris Buffer (pH 7.5)*			25 nmoles
17019	Biotin-14-dCTP *1 mM in Tris Buffer (pH 7.5)*			25 nmoles
17100	N6-Methyladenosine-Biotin conjugate			1 mg
20018	Annexin V-Biotin conjugate			100 tests
20605	Cal-520®-Biotin Conjugate	492	514	5x50 ug
23140	Phalloidin-Biotin Conjugate			300 Tests
45300	Biotin Styramide *Superior Replacement for Biotin Tyramide*			100 Slides

Fundamentals of Flow Cytometry

Flow cytometry is a powerful and flexible technique that can rapidly analyze multiple parameters of individual cells, within heterogeneous cell populations. Flow cytometers are utilized in a range of applications, from immuno-phenotyping, ploidy analysis and cell counting, to fluorescence-activated cell sorting (FACs), fluorescence expression analysis, and more. The flow cytometer performs this analysis by passing thousands of cells per second through a laser beam and capturing the fluorescence and scattered light that emerges from each cell. As the cells pass through, fluorescence data is collected and analyzed statistically by flow cytometry software to report cellular characteristics including size, complexity, phenotype and health (e.g. viability, proliferation and apoptotic states).

The principle behind fluorescence is that any fluorophore has a range of specific wavelengths at which it absorbs light energy. In absorption, high energy light excites fluorophores to a higher energy level called an excited state. Once this state is achieved, fluorophores return to a low energy ground state by releasing the excess energy in the form of light. This transition of energy is called fluorescence.

Fluorescence is an important feature of fluorophores as it is used to differentiate their "color", that is, the wavelength at which they emit fluorescent light. The ability to simultaneously utilize multiple fluorophore-conjugates with distinct and well-separated emission profiles permits multi-parametric analysis, which is the real power of flow cytometry. However, one of the biggest challenges in multi-parameter flow cytometry is knowing the correct combination of fluorophores to select in order to maximize assay sensitivity. Keep in mind, with the right combination one can minimize tedious compensation and spillover adjustments with no sacrifice to the quality and accuracy to the data collected. So how does one choose the optimal combination of fluorophores? The answer lies within the instrument.

What is a Flow Cytometer?

Schematically, the flow cytometer is comprised of three key systems: the fluidic, the optical and electronic system **(Figure 1).** The fluidic system is responsible for transporting cells. It passes cells individually through pressurized lines containing sheath fluid to the interrogation point where the laser intersects with the sample. The sample flow rate of the fluidic system can be manipulated in order to improve analysis. For example, a slow flow rate decreases the size of the sample stream, thereby increasing the accuracy and uniformity of sample detection.

The optical system is responsible for illumination and light collection within the flow cytometer. This system is comprised of excitation lasers, lenses and filters. The lasers ensure that

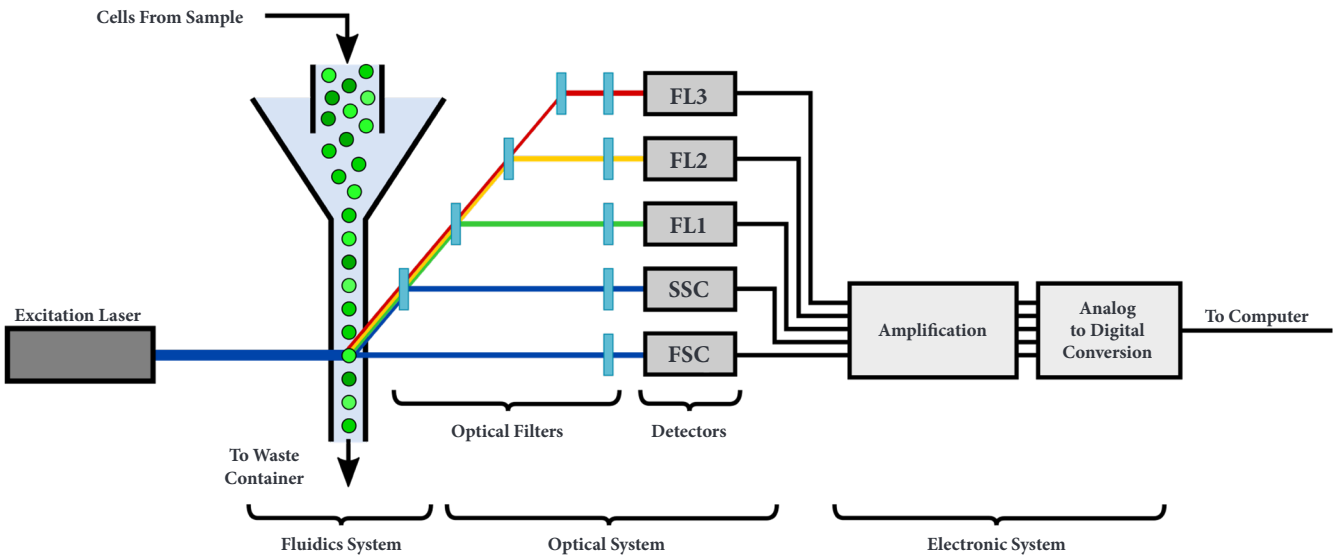

Figure 4.1 Flow cytometer schematics, illustrating the fluidics, opitcal and electronic systems.

cells in the interrogation point are excited with uniform light of a specific wavelength. For instance, argon lasers emit light at the 488 nm wavelength and can be used to excite fluorophores with a 488 nm absorption maximum such as iFluor™ 488 (Cat# 1023) and FITC (Cat# 135).

As the cells pass through the laser it emits fluorescence and scattered laser light at all angles. The collection optics – lenses and filters – serve to separate and direct the specific wavelengths of fluorescence and scattered laser light to the appropriate detectors. These detectors capture the emitted fluorescence and scattered laser lights, converts them into a photocurrent and passes it to the electronics system to be digitized and processed for subsequent analysis.

Since its introduction in the 1970s till today, flow cytometers have evolved significantly. Early designs consisted of single-laser cytometers that could only detect size. While todays cytometers feature multiple laser and filter configurations to facilitate multicolor analysis, with some cytometers capable of detecting up to 14 parameters simultaneously. Therefore, when choosing the appropriate fluorophores for multicolor analysis, instrument configuration – more importantly the lasers and filters – must be taken into careful consideration.

Choosing the Correct Fluorophore(s)

Before choosing any fluorophores, consider the following: (1) the type of laser(s) equipped in the flow cytometer, (2) the number of lasers present, (3) the excitation capabilities of each laser, and (4) how to appropriately configure filters. Step four is critical to assay sensitivity, setting a filter too wide may lead to excess background noise and false results.

Next, is to select the appropriate fluorophores. Criteria to consider when selecting fluorophores for multicolor flow cytometry include:

1. Strong fluorescence as characterized by high extinction coefficient and quantum yield
2. Excitation and emission maxima carefully matched to instrument laser and filter configuration
3. Well-separated emission profile to minimize spectral overlap and spillover
4. Large Stokes shift – critical in multiplexing analysis as the higher the value the greater the separation

Another good rule of thumb is to choose robust dye colors, as these will determine how prominent the signal is over the unstained cell population. However in some cases, it may make more sense to choose a dimer dye in order to avoid spillover and preserve resolution and sensitivity.

For assistance building multicolor flow cytometry panels use AAT Bioquest's Fluorescence Spectrum Viewer. This web-based tool allows users to specify instrument configuration (e.g. laser and filter setting) in order to check fluorophore compatibility. It can also be used to determine if two fluorophores have spectral overlap and if compensation will be required **(Figure 2).**

Table 1 is an example of a combination of fluorophores for a 6-color panel, these include: iFluor™ 488 (Cat# 1023), PE (Cat# 2558), PE-Cy5.5 (Cat# 2613), PE-Cy7 (Cat# 2616), APC (Cat# 2554) or iFluor™ 647 (Cat# 1031), and APC-Cy7 (Cat# 2625) (Table 1). These fluorophores are subsequently conjugated to an antibody target of choice and can be utilized in FACS or immuno-phenotyping. AAT Bioquest offers two easy-to-use antibody conjugation kits, ReadiLink™ Kits and Buccutite™ Kits, in a wide range of fluorescent labels, phycobiliproteins and tandem dyes compatible with any flow cytometer configuration. In addition, AAT Bioquest offers an array of high quality reagents for flow cytometry, some of which, including protocols, are highlighted in the following section.

Cell Surface and Intracellular Staining for Flow Cytometry

Flow cytometry is an extremely useful technique which can be used to analyze various cell surface and intracellular components. In the following section, we discuss flow cytometric reagents and protocols for cell surface and intracellular staining.

Staining Cell Surface Biomarkers

Surface antigens are expressed on the membrane of the cells and thus easily accessible. A majority of flow cytometric applications have focused on characterization via cell surface antigens to define cell subtypes as well as functions using fluorescent labeled antibodies. Because these surface antigens are easily accessible by antibodies, they can be readily stained without permeabilization steps. One advantage of exploiting surface antigens is that live cells can be subjected to cell sorting

models, such as FACS, and used for downstream applications.

When cell surface staining, consider the following:

1. Prepare the desired biological cells according to the appropriate protocol.
2. Wash cells with PBS buffer to remove excess cell culture medium.
3. Reduce non-specific interactions by treating cells with a blocking agent such as 1% fetal bovine serum or BSA.
4. After blocking, stain cell surface antigens with fluorescently labeled antibody conjugates.
5. Wash cells at least twice with desired buffer.
6. Re-suspend cells in ice-cold PBS and analyze using flow cytometer

Figure 3, illustrates cell surface staining utilizing the aforementioned considerations. In this example, peripheral blood mononuclear cells (PBMCs) were labeled with cell surface antibody CD45 conjugated with phycoerythrin (PE). Cells were incubated with Fc StainX (Biolegend) for 20 minutes at room temperature to block non-specific interaction. Following blocking, cells were incubated with CD45-PE conjugates for 20 minutes on ice, protected from light. Cells were then washed with PBS buffer, re-suspended in stain buffer and analyzed using an ACEA NovoCyte flow cytometer.

Staining for intracellular targets

Intracellular targets can also be stained and detected using flow cytometry. However, since these targets are located inside the cells, intracellular staining requires fixation and permeabilization steps to allow for target interactions (e.g. antigen-antibody). Fixation can be achieved using low concentrations of formaldehyde or alcohol, while permeabilization requires treating cell with detergents such as saponin, Triton-X-100, or organic solvents. When staining intracellularly, it is also important to consider target location (e.g. cytosol or nucleus), as this will determine which protocol and buffer system is optimal.

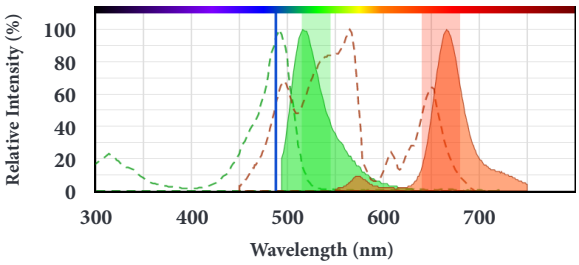

Figure 4.2 Excitation and emission spectrum of iFluor™ 488 (Cat# 1023, green) and PE-Cy5 tandem (Cat# 2610, red) illustrating the clear spectral separation that enables multicolor analysis. Both iFluor™ 488 and PE-Cy5 can be efficiently excited by the 488 nm laser.

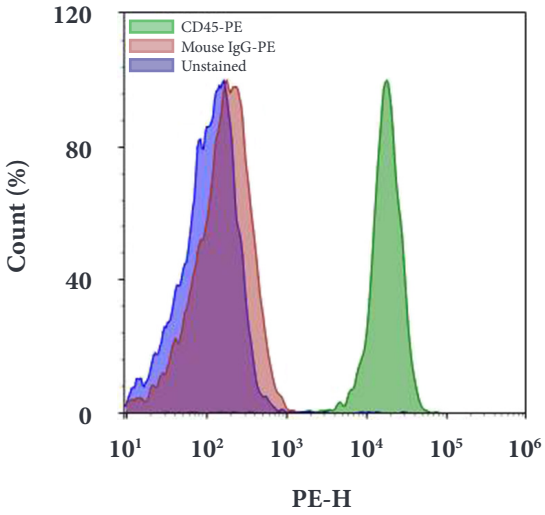

Figure 4.3 Single parameter histogram of lymphocytes isolated from PBMCs. The PBMCs were labelled with CD45 antibody tagged with PE (1µg/mL) for cell surface antigens. The labelled cells were gated on lymphocytes and analysed by ACEA NovoCyte flow cytometer.

Table 4.1 Six color flow cytometery panel.

Cat#	Product Name	Ex (nm)	Em (nm)	Stokes Shift (nm)
1023	iFluor™ 488 succinimidyl ester	491	518	27
2558	PE [R-Phycoerythrin] *CAS 11016-17-4*	565	575	10
2613	PE-Cy5.5 Tandem	565	700	135
2616	PE-Cy7 Tandem	565	780	215
2554	APC [Allophycocyanin]	651	662	11
1031	iFluor™ 647 succinimidyl ester	649	664	15
2625	APC-Cy7 Tandem	651	780	129

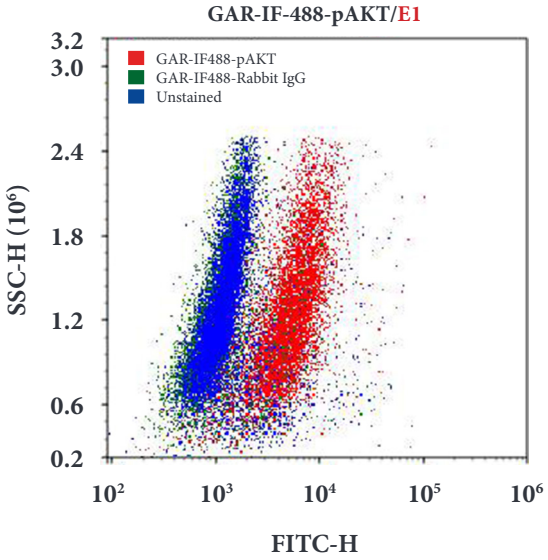

Figure 4.4 Single parameter histogram of lymphocytes isolated from PBMCs. The PBMCs were labelled with CD45 antibody tagged with PE (1µg/mL) for cell surface antigens. The labelled cells were gated on lymphocytes and analysed by ACEA NovoCyte flow cytometer.

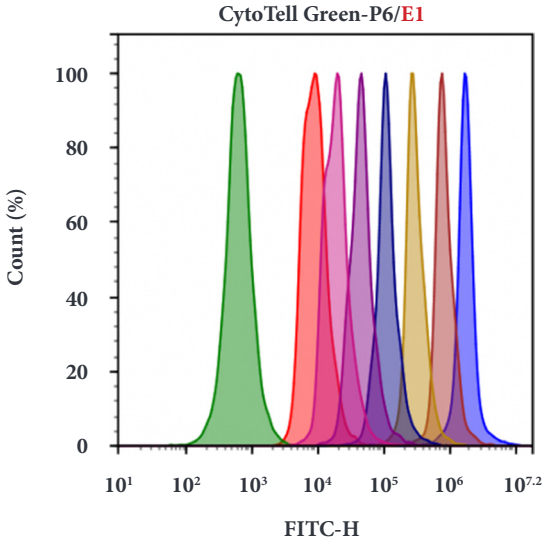

Figure 4.5 Cell tracking assay with CytoTell™ Green. Jurkat cells (2 x 106) were plated and stained with CytoTell™ Green dyes on day 0. The cells were passed serially at 1:1 ratio and passaged for 9 days. Fluorescence intensity was measured using NovoCyte flow cytometer from ACEA Biosciences, Inc. Successive generations were represented by different colors.

Figure 4, illustrates intracellular staining utilizing iFluor 488-secondary conjugates. In this example, Jurkat cells were fixed with 100% Methanol for 30 minutes at -20oC. Cells were then washed with wash buffer and blocked using PBST containing 1% BSA for 30 minutes at room temperature to reduce non-specific binding of the antibodies. After blocking, cells were incubated with p-Akt or Rabbit IgG for 60 minutes at room temperature, and then washed with wash buffer. Next, iFluor 488 goat anti-rabbit secondary antibodies were added for 30 minutes at room temperature, protected from light. Cells were then washed with wash buffer, re-suspended in stain buffer and analyzed using an ACEA NovoCyte flow cytometer

Tracking Cell Proliferation by Flow Cytometry

While regulated cell proliferation promotes healthy growth and development, dysregulation to the cell cycle is a hallmark indication of cancer and autoimmune disease. The use of high-throughput, fluorescence-based methods such as flow cytometry are particularly useful to the study of proliferation and in the screening novel cancer therapies. When choosing a cell proliferation tracer there is several criteria to consider: (1) the cell tracer must be non-toxic to cells, (2) the cell tracer must show minimal dye transfer between adjacent cells, and (3) the cell tracer must exhibit bright and stable fluorescence in labeled cells.

The CytoTell dyes (Figure 5) were created with the aforementioned criteria in mind. Similar to CFSE, CytoTell™ dyes are cell-permeable indicators that react with cytoplasmic proteins through lysine residues and other amine sources, producing high fluorescent, cell-impermeant proteins. The resulting covalent bond ensures CytoTell™ dyes are not transferred to adjacent cells and are well-retained in cells for several generations. As cells divide, CytoTell™ fluorescent proteins are equally distributed between daughter cells during cell division, and each new generation of cells is marked by a fluorescence intensity half that of its parents, which is readily detectable by flow cytometry.

Compared to CFSE, CytoTell™ dyes have minimal cytotoxicity and better cellular retention. CytoTell™ dyes, other than CytoTell™ Green, are excellent for multicolor applications with either GFP cell lines or iFluor 488™-labeled antibodies.

References

1. Adan, A., Alizada, G., Kiraz, Y., Baran, Y. & Nalbant, A. Flow cytometry: basic principles and applications. Critical Rev. Biotechnol. 37, 163–176.

2. Davies D. (2010). Cell sorting by flow cytometry. In: Macey MG, ed. Flow cytometry: principles and applications. Totowa (NJ): Humana Press, 257–76
3. Macey MG. (2010). Principles of flow cytometry. Flow cytometry: principles and applications In: Macey MG, ed. Totowa (NJ): Humana Press, 1–15
4. Wlodkowic D, Skommer J, Darzynkiewicz Z. (2009). Flow cytometry-based apoptosis detection. Methods Mol Biol, 559, 19–32
5. Errante, Paolo. (2016). Flow cytometry: a literature review. 10.13140/RG.2.1.2461.0969
6. Laerum, O. D. and Farsund, T. (1981), Clinical application of flow cytometry: A review. Cytometry, 2: 1-13. doi:10.1002/cyto.990020102
7. Wood, J.C.S. and Hoffman, R.A. (1998) Evaluating fluorescence sensitivity on flow cytometers: an overview. Cytometry, 33: 256-259.
8. Van Dilla, M.A., Dean, P.N., Laerum, O.D. and Melamed, M.R. (eds.) (1985). Flow cytometry instrumentation and data analysis. Academic Press, Orlando.
9. Wood, J.C.S. (1993). Clinical Flow Cytometry Instrumentation. In: Clinical Flow Cytometry. Principles and Applications. (eds. Bauer, K.D, Duque, R.E. and Shankey, T.V.), pp. 71-92. Williams and Wilkins, Baltimore.

Product Ordering Information

Table 4.2 Product ordering information for CytoTell™ dyes, ReadiLink™ Antibody Labeling Kits, and Buccutite™ Protein Labeling Kits.

Cat#	Product Name	Ex (nm)	Em (nm)	Size
22251	CytoTell™ Blue	403	454	500 Tests
22253	CytoTell™ Green	511	525	500 Tests
22240	CytoTell™ UltraGreen	492	519	500 Tests
22255	CytoTell™ Red 650	628	643	500 Tests
22261	CytoTell™ Red 590	573	588	500 Tests
22257	CytoTell™ Orange	542	556	500 Tests
1220	ReadiLink™ Rapid iFluor™ 350 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	345	442	2 Labelings
1227	ReadiLink™ Rapid iFluor™ 555 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	552	567	2 Labelings
1230	ReadiLink™ Rapid iFluor™ 594 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	592	614	2 Labelings
1235	ReadiLink™ Rapid iFluor™ 647 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	654	674	2 Labelings
1240	ReadiLink™ Rapid iFluor™ 680 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	682	701	2 Labelings
1245	ReadiLink™ Rapid iFluor™ 700 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	693	713	2 Labelings
1250	ReadiLink™ Rapid iFluor™ 750 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	753	779	2 Labelings
1255	ReadiLink™ Rapid iFluor™ 488 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	491	514	2 Labelings
1260	ReadiLink™ Rapid iFluor™ 633 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	638	655	2 Labelings
1265	ReadiLink™ Rapid iFluor™ 790 Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg Antibody Per Reaction*	782	811	2 Labelings
1311	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	651	662	2 Labelings
1312	Buccutite™ Rapid PE Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	575	2 Labelings

Table 4.2 Cont. Product ordering information for CytoTell™ dyes, ReadiLink™ Antibody Labeling Kits, and Buccutite™ Protein Labeling Kits.

Cat#	Product Name	Ex (nm)	Em (nm)	Size
1313	Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	651	662	2 Labelings
1316	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	565	700	2 Labelings
1317	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	565	780	2 Labelings
1318	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	565	600	2 Labelings
1319	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	651	713	2 Labelings
1320	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	651	700	2 Lalelings
1321	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	651	780	2 Labelings
1322	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	565	674	2 Labelings
1325	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*	482	677	2 Labelings
1340	Buccutite™ Rapid PE-Cy5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	674	2 Labelings
1341	Buccutite™ Rapid PE-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	700	2 Labelings
1342	Buccutite™ Rapid PE-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	780	2 Labelings
1343	Buccutite™ Rapid PE-Texas Red Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	565	600	2 Labelings
1347	Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	651	713	2 Labelings
1350	Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	651	700	2 Labelings
1351	Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	651	780	2 Labelings
1353	Buccutite™ Rapid PerCP Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction*	482	677	2 Labelings

ReadiUse™ Lyophilized Phycobiliproteins

Phycobiliproteins are a family of photosynthetic light-harvesting proteins derived from microalgae and cyanobacteria. These proteins have covalently attached linear tetrapyrrole groups, known as phycobilins, which play a critical role in capturing light energy. In microalgae and cyanobacteria, energy absorbed by these phycobilins is efficiently transferred via fluorescence resonance energy transfer (FRET), to chlorophyll pigments for use in photosynthetic reactions.

Compared to chemically synthetic fluorescent dyes, phycobiliproteins emit intense fluorescence signals due to their relatively high fluorescence quantum yields and extinction coefficients. Because their fluorescence is not quenched by biomolecules, phycobiliproteins can serve as valuable fluorescence tags in numerous applications. Phycobiliproteins-conjugated to biological molecules (e.g. immunoglobulin, protein A or streptavidin) have found great success in flow cytometry, fluorescence activated cell sorting (FACS), histochemistry, imaging and to a limited degree reactive oxygen species detection. Two main classes of phycobiliproteins are commercially available phycoerythrin (PE) and allophycocyanin (APC).

Phycoerythrin (PE)

PE (Cat# 2558) exhibits an intensely bright yellow-orange fluorescence with an extinction coefficient (ε) of 1,960,000 cm-1M-1 and a quantum yield (Φ) of 0.84. In comparison to Cy3® (Cat# 141), which has an extinction coefficient (ε) of 150,000 cm-1M-1 and a quantum yield (Φ) of 0.24, PE is significantly brighter. Its absorbance spectrum, which is characterized by three absorption bands, shows a primary absorbance peak at 565 nm with two secondary peaks at 496 nm and 545 nm. The relative prominence of the secondary peaks varies significantly among PE's derived from different species.

Phycoerythrin is available in two forms. R-phycoerythrin (R-PE), which is isolated from RhodophytaI, and B-phycoerythrin (B-PE), which is isolated from Bangiales. Both B-PE and R-PE are comprised of three types of subunits: α (~20,000 daltons), β (~20,000 daltons) and γ (~30,000 daltons). While they share similar subunit structure, the chromophore content of their subunits differs causing differences in the relative intensities of their absorbance peaks.

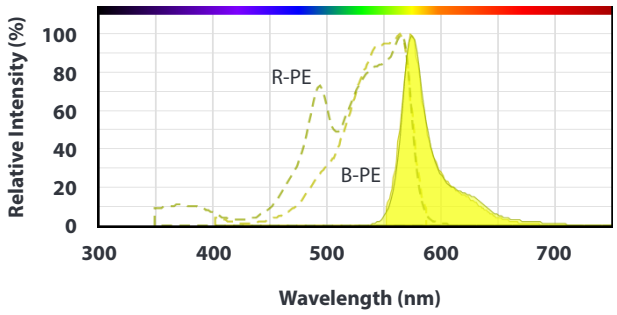

Figure 5.1 Comparison of absorbance and emission spectra of R-PE and B-PE.

Table 5.1 Spectral Properties of R-PE and B-PE.

Properties	R-PE	B-PE
Common Subunits	$(\alpha\beta)_{6\gamma}$	$(\alpha\beta)_{6\gamma}$
MW	240000	240000
Absorption maximum	565 nm	545nm
Additional Absorption peak	498 nm	563nm
Emission maximum	573 nm	572nm
Extinction Coefficient (ε)	1.96 x 10^6 $M^{-1}cm^{-1}$	2.41 x 10^6 $M^{-1}cm^{-1}$
Quantum Yield (QY)	0.84	0.98
Brightness (ε x QY)	1.65 x 10^6 $M^{-1}cm^{-1}$	2.36 x 10^6 $M^{-1}cm^{-1}$
Specifications	A566/A280 ≥ 5.0	A545/A280 ≥ 5.5
	A566/A498 < 1.5	A620/A546 ≤ 0.01
	A620/A566 < 0.01	

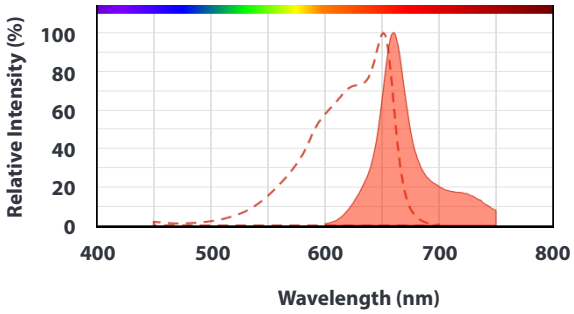

Figure 5.2 Absorbance and emission spectra of APC.

Table 5.2 Spectral Properties of APC.

Properties	APC
Common Subunits	$(\alpha\beta)_3$
MW	105000
Absorption maximum	651 nm
Additional Absorption peak	625 nm
Emission maximum	662 nm
Extinction Coefficient (ε)	7.3 x 10^5 $M^{-1}cm^{-1}$
Quantum Yield (QY)	0.68
Brightness (ε x QY)	4.9 x 10^5 $M^{-1}cm^{-1}$
Specifications	A650/A260 ≥ 1.25
	A650/A280 < 4.5
	Cross-link Ratio ≥ 1.0

Allophycocyanin (APC)

APC (Cat# 2554) exhibits a bright far-red fluorescence with an extinction coefficient of 700,000 cm-1M-1 and a quantum yield (Φ) of 0.68. In comparison to Cy5® (Cat# 151), which has an extinction coefficient (ε = 250,000 cm-1M-1) and quantum yield (Φ = 0.20) of Cy5®, APC is significantly burgher. It has as a primary absorbance maximum at 652 nm with a secondary maximum at 625 nm. APC has a fluorescence emission maximum at 662 nm, which is in the red region of the visible spectrum.

ReadiUse™ PE & APC

In order to use PE or APC for flow cytometry, the fluorescenct protein must first be conjugated to an antibody. However, commercially available phycobiliproteins come concentrated in an ammonium sulfate suspension. Although both exhibit good long-term stability, they require tedious purification processes, such as dialysis, sulfate precipitation or gel filtration chromatography, to remove the ammonium sulfate impurities prior to antibody conjugation. Once purified PE and APC must be stored refrigerated, not frozen, and consumed within one month for optimal results.

AAT Bioquest has developed an innovative technique for the lyophilization of purified phycobiliproteins. Our line of ReadiUse™ phycobiliproteins are supplied as lyophilized powders free of ammonium sulfate. Removal of this strong precipitating agent eliminates purification processes, and facilitates the rapid conjugation of PE or APC to antibodies and other proteins.

The following is an overview of a protocol for ReadiUse™ PE (Cat# 2500) using Buccutite™ Rapid Protein Crosslinking Kit (Cat# 1315):

1. Reconstitute ReadiUse™ PE in H2O
2. Activate PE and antibody with Buccutite™ conjugation linkers
3. Mix Buccutite activated PE and activated antibody
4. Purify Conjugates using desalting column

Comparative Analysis: ReadiUse™ vs The Competition

In the present study, lyophilized ReadiUse™ PE (Cat# 2500) and ReadiUse™ APC (Cat# 2503) (AAT Bioquest) were stored under various conditions and subsequently tested for performance against traditional phycobiliproteins, suspended in ammonium sulfate. Prior to testing, traditional phycobiliproteins were purified to remove ammonium sulfate. Comparisons were made for spectral property, fluorescence quantum yields and labeling efficiencies.

Storage Conditions Tested

ReadiUse™ PE and ReadiUse™ APC were aliquoted into 1 mg vials and stored at four different temperatures for duration of two months. Temperatures include:

1. Fresh – Freshly purified phycobiliproteins suspended in ammonium sulfate (not stored, prepared on day of experiemnt)
2. L – 4°C – Lyophilized powder, ReadiUse™ PE and ReadiUse™ APC stored at 2 – 8°C
3. L – RT – Lyophilized powder, ReadiUse™ PE and ReadiUse™ APC stored at room temperature
4. L – (-20°C) – Lyophilized powder, ReadiUse™ PE and ReadiUse™ APC stored at < - 15°C
5. L – (-65°C) – Lyophilized powder, ReadiUse™ PE and ReadiUse™ APC stored a < -65°C

Preparing PE & APC

On test day, phycobiliproteins saturated in ammonium sulfate solution were purified using dialysis to remove ammonium sulfate impurities. ReadiUse™ PE and ReadiUse™ APC were removed from storage and reconstituted with 100 µL H2O for a final concentration of 10 mg/mL.

ReadiUse™ PE Spectral Properties

The absorbance and emission spectra of ReadiUse™ PE and freshly purified PE in PBS, were measured under identical conditions and concentrations. The spectra comparison data is summarized in Table 3, Table 4 and Figure 3. Data illustrates that ReadiUse™ PE satisfies all the specifications of PE, is very stable and can be stored from RT to -65oC for ~2 months with little variation in excitation, emission and quantum yields.

ReadiUse™ APC Spectral Properties

The absorbance and emission spectra of ReadiUse™ APC and freshly purified APC in PBS, were measured under identical conditions and concentrations. The spectra comparison data is summarized in Table 5, Table 6 and Figure 4. Data illustrates that ReadiUse™ APC satisfies all the specifications of APC, is

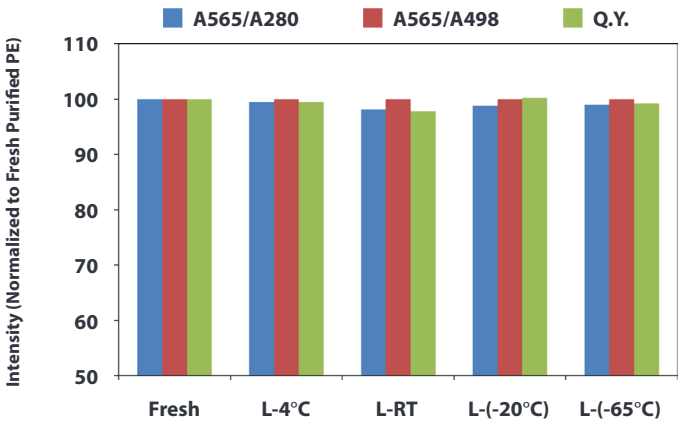

Figure 5.3 Comparison of lyophilized ReadiUse™ PE and liquid form PE. Absorbance ratios of 565 to 280 nm and 565 to 498 nm were compared and the relative emission intensity at the same concentration were also measured with 490 nm excitation and plotted in the figure.

Table 5.3 Spectral properties comparison of ReadiUse™ PE and ammonium-sulfate supplied PE.

PE	Fresh (Purified)	L - 4°C	L - RT	L - (-20°C)	L - (-65°C)	Specification
A565/A280	5.83	5.72	5.72	5.76	5.77	>5.6
A565/A498	1.45	1.45	1.45	1.45	1.45	<1.5
A620/A565	0	0	0.003	0.002	0.002	<0.005
Emission Max	574 nm	574 nm	574 nm	574 nm	574 nm	574 nm

Table 5.4 Quantum yield of ReadiUse™ PE and ammonium-sulfate supplied PE.

Properties	Fresh(Purified)	L - 4°C	L - RT	L - (-20°C)	L - (-65°C)
Excitation Wavelength	490 nm	490 nm	490 nm	490 nm	490 nm
Emission Wavelength	574 nm	574 nm	574 nm	574 nm	574 nm
Relative Quantum Yield	100%	99.5%	97.8%	100%	99.3%

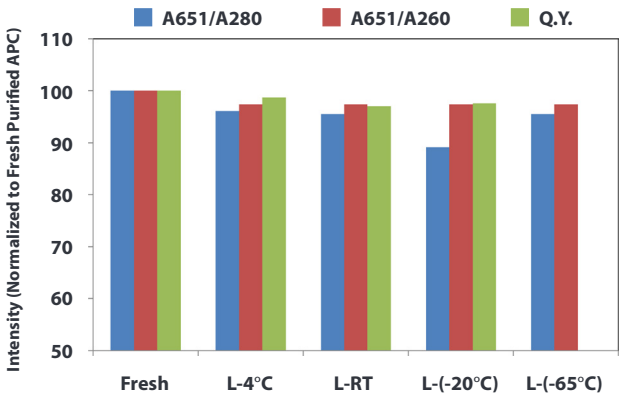

Figure 5.4 Comparison of lyophilized ReadiUse™ APC and liquid form APC. Spectra properties were compared and relative emission intensity at the same concentration were measured with 630 nm excitation.

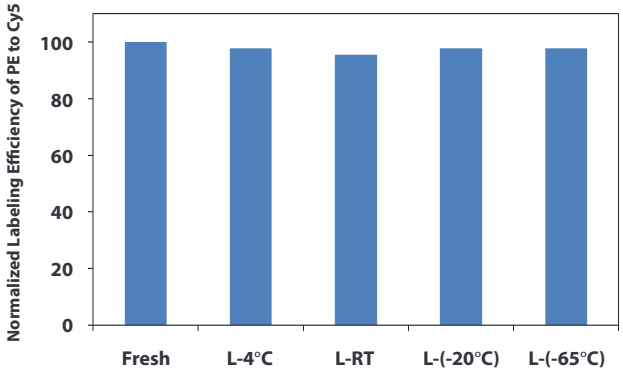

Figure 5.5 Comparison of lyophilized ReadiUse™ PE and Fresh PE labeling efficiencies.

very stable and can be stored from RT to -65°C for ~2 months with little variation in excitation, emission and quantum yields.

Labeling Efficiency of Lyophilized ReadiUse™ PE and Liquid Form PE

The labeling efficiency of ReadiUse™ PE is another important factor to examine as it directly affects the conjugate's fluorescence yield. For example, a common application of PE is to modify it with Cy5 to produce a PE/Cy5 tandem dye. Over-labeling of Cy5 onto PE will significantly reduce fluorescence yield due to anomalously strong quenching.

Labeling efficiency can be determined by calculating the degree of labeling, which is the molar ratio of dye to protein (PE) after conjugation. The reactivity of ReadiUse™ PE was validated using Cy5 conjugation reaction. ReadiUse™ PE, stored at different temperatures (4°C, RT, -20°C and -65°C for 2 months), were reconstituted with 100 µL of H_2O for a final concentration of 10 mg/mL. A Cy5, SE (Cat# 151) dye working solution was prepared and added to ReadiUse™ PE solution. The conjugation reaction was done at room temperature for 60 minutes with stirring. Freshly prepared PE was conjugated to Cy5 in the same manner and used as a positive control Figure 5.

Labeling Efficiency of Lyophilized ReadiUse™ APC and Liquid Form APC

The labeling efficiency of ReadiUse™ APC was validated using Cy7 conjugation reaction. ReadiUse™ APC, stored at different temperatures (4°C, RT, -20°C and -65°C for 2 months), were reconstituted with 100 µL of H_2O for a final concentration of 10 mg/

Table 5.5 Spectral properties comparison of ReadiUse™ APC and ammonium-sulfate supplied APC.

APC	Fresh (Purified)	L - 4°C	L - RT	L - (-20°C)	L - (-60°C)	Specification
A651/A280	5.18	4.98	4.95	4.62	4.95	>4.6
A651/A620	1.57	1.53	1.53	1.53	1.53	>1.5
Emission Max	661.00 nm	660.00 nm	660.00 nm	660.00 nm	660.00 nm	

Table 5.6 Quantum yield of ReadiUse™ APC and ammonium-sulfate supplied APC.

Properties	Fresh (Purified)	L - 4°C	L - RT	L - (-20°C)	L - (-65°C)
Excitation (nm)	630 nm	630 nm	630 nm	630 nm	630 nm
Emission (nm)	661.01 nm	660.00 nm	660.00 nm	660.00 nm	660.00 nm
Relative Quantum Yield	100%	98.7%	97%	97%	97%

mL. A Cy7, SE (Cat# 170) dye working solution was prepared and added to ReadiUse™ APC solution. The conjugation reaction was done at room temperature for 60 minutes with stirring. Freshly prepared APC was conjugated to Cy7 in the same manner and used as a positive control Figure 6.

The performance of ReadiUse PE-Cy5 tandems and ReadiUse™ APC-Cy7 tandems were tested in HL-60 cells. ReadiUse™ PE-Cy5 tandems and ReadiUse™ APC-Cy7 tandems were conjugated to GXM IgG using Buccutite™ Rapid Protein Conjugation kit (Cat# 1315). Conjugates were also prepared with freshly purified PE-Cy5 and PE-Cy7 tandems using Buccutite™ conjugation kit, and used as a positive control. The results indicated that ReadiUse™ PE-Cy5 and ReadiUse APC-Cy7 conjugates were equivalent in performance to freshly purified PE-Cy5 and APC-Cy7 conjugates.

Conclusion

AAT Bioquest's ReadiUse™ PE and ReadiUse™ APC are on par with commercially available products. Our lyophilized phycobiliproteins are stable at different conditions, such as RT, 4°C and -20°C and -65°C without changes in spectra properties, labeling efficiency and performance. In addition, ReadiUse™ PE and ReadiUse™ APC ammonia sulfate-free powders, do not require any purification (e.g. dialysis or desalting), significantly reduces assay time, increases the consistency in quality and the flexibility in reconstitution concentration, and also decreases the shipping cost.

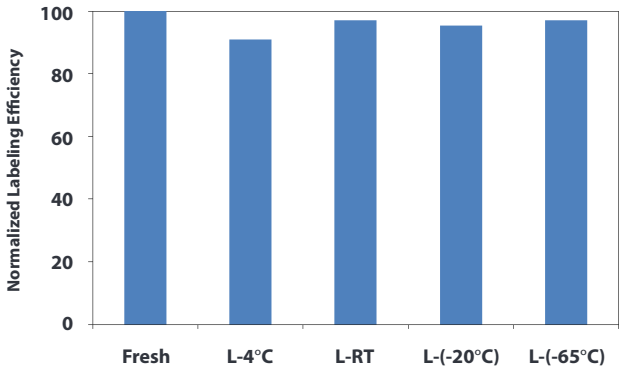

Figure 5.6 Comparison of lyophilized ReadiUse™ APC and Fresh APC labeling efficiencies.

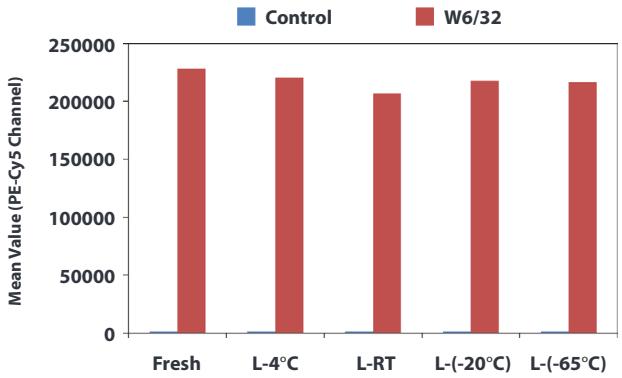

Figure 5.7 Comparison the performance of GXM IgG-PE/Cy5 conjugates with ReadiUse™ PE-Cy5 tandems and freshly purified PE-Cy5 tandems. HL-60 cells were stained with or without w6/32 antibody (1ug/ml) for 30 min, and followed by GXM IgG-PE/Cy5 (10ug/ml) for 30min. GXM IgG-PE/Cy5 conjugates were prepared through the same Buccutite™ reaction.

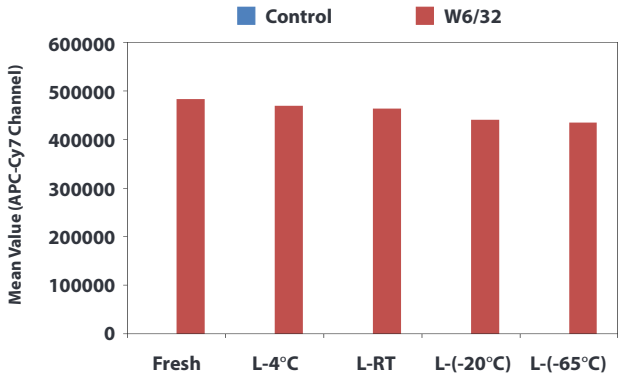

Figure 5.8 Performance of GXM IgG-APC/Cy7 conjugate prepared with ReadiUse™ APC-Cy7 tandems and freshly purified APC-Cy7 tandems. HL-60 cells were stained with or without w6/32 antibody (1ug/ml) for 30min, and followed by GXM IgG-APC/Cy7 (5ug/ml) for 30min. GXM IgG-APC/Cy7 conjugates were prepared through the same Buccutite™ reaction.

Table 5.7 Product ordering information for ReadiUse™ PE, ReadiUse™ APC and ReadiUse™ Tandem dyes.

Cat#	Product Name	Ex (nm)	Em (nm)	Size
2500	ReadiUse™ PE [R-Phycoerythrin] *Ammonium Sulfate-Free*	565	575	1 mg
2501	ReadiUse™ PE [R-Phycoerythrin] *Ammonium Sulfate-Free*	565	575	10 mg
2503	ReadiUse™ CL-APC [Cross linked-Allophycocyanin] *Ammonium Sulfate-Free*	651	662	1 mg
2504	ReadiUse™ CL-APC [Cross linked-Allophycocyanin] *Ammonium Sulfate-Free*	651	662	10 mg
2560	ReadiUse™ Preactivated PE	565	575	1 mg
2561	ReadiUse™ Preactivated APC	651	713	1 mg
2570	ReadiUse™ Preactivated APC-iFluor™ 700 Tandem	651	713	1 mg
2571	ReadiUse™ Preactivated APC-iFluor™ 750 Tandem	651	779	1 mg
2577	ReadiUse™ Preactivated PE-iFluor™ 647 Tandem	565	674	1 mg
2578	ReadiUse™ Preactivated PE-iFluor™ 750 Tandem	565	779	1 mg
2580	ReadiUse™ Preactivated PE-Cy5 Tandem	565	670	1 mg
2581	ReadiUse™ Preactivated PE-Cy5.5 Tandem	565	700	1 mg
2582	ReadiUse™ Preactivated PE-Cy7 Tandem	565	780	1 mg
2583	ReadiUse™ Preactivated PE-Texas Red Tandem	565	600	1 mg
2584	ReadiUse™ Preactivated PE-iFluor™ 594 Tandem	565	600	1 mg
2586	ReadiUse™ Preactivated APC-Cy5.5 Tandem	651	700	1 mg
2587	ReadiUse™ Preactivated APC-Cy7 Tandem	651	780	1 mg

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