Cell Analysis Probes & Assay Kits

FLOW CYTOMETRY · FLUORESCENCE IMAGING · HIGH CONTENT ANALYSIS

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Stain IT™
Tide Fluor™
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Custom Products and Services

Our Technologies

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

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

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

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

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

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

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

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

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

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

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

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

Our Services

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

Custom Assay Design and Development

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

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

Custom Conjugation

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

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

Custom Screening

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

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

Custom Synthesis of Fluorophores and Luminophores

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

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Tools for Fluorescence Imaging & Flow Cytometry

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imaging & flow cytometry tools at-a-glance

	Fluorescence Imaging	Flow Cytometry
Anti-Fading Reagents	Fluoroquest™ Reagents	
Conjugation Tools	ReadiLink™ Kits	ReadiLink™ Kits
Fluorescent Tags	iFluor™ Dyes trFluor™ Dyes	iFluor™ Dyes mFluor™ Dyes
Secondary Antibodies	iFluor™ Conjugates trFluor™ Conjugates	iFluor™ Conjugates mFluor™ Conjugates
Streptavidin Conjugates	iFluor™ Conjugates trFluor™ Conjugates	iFluor™ Conjugates Phycobiliprotein Conjugates

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Tools for Fluorescence Imaging & Flow Cytometry

2.1 iFluor™ Dyes, Optimized for Labeling Antibodies

AAT Bioquest is rapidly expanding our product lines to meet your constantly changing research needs. We have been developing fluorescent dyes to solve various limitations with the existing fluorescent labeling reagents while offering classic fluorescent labeling reagents with high purity and competitive price to help you to get more research done with less money.

iFluor™ dyes are the products of our recent R&D efforts. They are a series of excellent fluorescent labeling dyes that span the full UV-visible and near IR spectrum. All the iFluor™ dyes have excellent water solubility. Their hydrophilic property makes their protein conjugation readily performed in aqueous media, minimizing the use of organic solvents. The resulted conjugates are resistant to precipitation during storage. iFluor™ dyes also have much better labeling performance than the classic fluorescent labeling dyes such as FITC, TRITC, Texas Red®, Cy3®, Cy5® and Cy7®. Some of our iFluor™ dyes significantly outperform Alexa Fluor® labeling dyes on certain antibodies.

Key Features of iFluor™ Dyes

- *Excellent water solubility*
- *Available in a variety of fluorescence colors*
- *Their conjugates exhibit more intense fluorescence than other spectrally similar conjugates of classic fluorescent dyes*
- *More photostable than the classic fluorescent dyes*
- *Absorption spectra match the principal output wavelengths of common excitation sources*
- *Robust and highly fluorescent over a broad pH range*

Table 2.1 iFluor™ Dye Equivalents of Common Dyes

If you are using	Try this iFluor™ dye
Alexa Fluor® 350, AMCA, DyLight™ 350	iFluor™ 350
Alexa Fluor® 405, DyLight™ 405	iFluor™ 405
Alexa Fluor® 488, Cy2®, FITC, DyLight™ 488	iFluor™ 488
Alexa Fluor® 514	iFluor™ 514
Alexa Fluor® 532	iFluor™ 532
Alexa Fluor® 555, Cy3®, DyLight™ 550, TRITC	iFluor™ 555
Alexa Fluor® 594, DyLight™ 594, Texas Red®	iFluor™ 594
Alexa Fluor® 633, DyLight™ 633	iFluor™ 633
Alxea Fluor® 647, Cy5®, DyLight™ 650	iFluor™ 647
Alexa Fluor® 680, Cy5.5®, IRDye® 700, DyLight™ 680	iFluor™ 680
Alexa Fluor® 700	iFluor™ 700
Alexa Fluor® 750, Cy7®, DyLight™ 750	iFluor™ 750
Alexa Fluor® 790, DyLight™ 800, IRDye® 800	iFluor™ 790

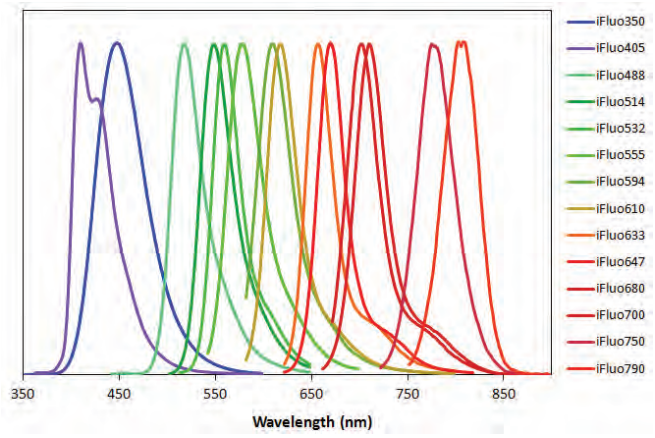

Figure 2.1. The normalized fluorescence spectra of iFluor™ dyes in PBS buffer (pH 7.2)

Table 2.2 Amine-Reactive iFluor™ Dyes for Labeling Antibodies

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
1020	iFluor™ 350 succinimidyl ester	1 mg	345	442
1021	iFluor™ 405 succinimidyl ester	1 mg	401	420
1023	iFluor™ 488 succinimidyl ester	1 mg	491	514
1024	iFluor™ 514 succinimidyl ester	1 mg	518	542
1025	iFluor™ 532 succinimidyl ester	1 mg	542	558
1028	iFluor™ 555 succinimidyl ester	1 mg	559	569
1029	iFluor™ 594 succinimidyl ester	1 mg	592	614
1038	iFluor™ 610 succinimidyl ester	1 mg	605	627
1030	iFluor™ 633 succinimidyl ester	1 mg	638	655
1031	iFluor™ 647 succinimidyl ester	1 mg	654	674
1035	iFluor™ 680 succinimidyl ester	1 mg	682	701
1036	iFluor™ 700 succinimidyl ester	1 mg	693	713
1037	iFluor™ 750 succinimidyl ester	1 mg	753	779
1368	iFluor™ 790 succinimidyl ester	1 mg	782	811

Table 2.3 Thiol-Reactive iFluor™ Dyes for Labeling Antibodies

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
1060	iFluor™ 350 maleimide	1 mg	345	442
1062	iFluor™ 488 maleimide	1 mg	491	514
1063	iFluor™ 555 maleimide	1 mg	559	569
1065	iFluor™ 647 maleimide	1 mg	654	674
1066	iFluor™ 680 maleimide	1 mg	682	701
1067	iFluor™ 700 maleimide	1 mg	693	713
1068	iFluor™ 750 maleimide	1 mg	753	779
1366	iFluor™ 790 maleimide	1 mg	782	811

2.2 mFluor™ Dyes, Optimized for Labeling Antibodies

mFluor™ dyes are a series of excellent fluorescent labeling dyes that span the full UV-visible spectrum. All the mFluor™ dyes are designed to be maximally excited by one of the major light sources equipped in flow cytometers (such as the violet laser at 405 nm, blue laser at 488 nm, green laser at 532 nm, yellow laser at 561 nm and red laser at 633 nm). They are excellent alternatives to the phycobiliprotein-based tandems that are quite difficult to be coupled to an antibody or other biomolecules. However, mFluor™ dyes are dimmer than RPE, APC and PerCP.

Key Features of mFluor™ Dyes

- *Available in a variety of reactive forms*
- *Available in a few distinct fluorescence colors*
- *Much easier for conjugation, giving much higher conjugation yields than tandems*
- *Maximally excited by one of the major light sources (405, 488, 532, 561 and 633 nm) used in flow cytometers*
- *Much more photostable than the phycobiliprotein tandems*
- *Robust and highly fluorescent over a broad pH range with little pH sensitivity*

Table 2.4 mFluor™ Dye Equivalents of Common Dyes

If you are using	Try this mFluor™ dye
Pacific Blue®	mFluor™ Violet 450
AmCyan	mFluor™ Violet 510
Pacific Orange®, Krome Orange™	mFluor™ Violet 540
RPE	mFluor™ Blue 570
APC-Cy5.5®, APC-Alexa Fluor® 680, APC-Alexa Fluor® 700 tandem	mFluor™ Red 700
APC-Cy7®, APC-Alexa Fluor® 750, APC-H7 tandem	mFluor™ Red 780

Table 2.5 Amine-Reactive mFluor™ Dyes for Labeling Antibodies

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
1160	mFluor™ Blue 570 SE	1 mg	553	570
1165	mFluor™ Green 620 SE	1 mg	523	617
1190	mFluor™ Red 700 SE	1 mg	657	700
1191	mFluor™ Red 780 SE	1 mg	629	780
1150	mFluor™ Violet 450 SE	1 mg	403	454
1151	mFluor™ Violet 510 SE	1 mg	414	508
1152	mFluor™ Violet 540 SE	1 mg	399	550
1170	mFluor™ Yellow 630 SE	1 mg	611	630

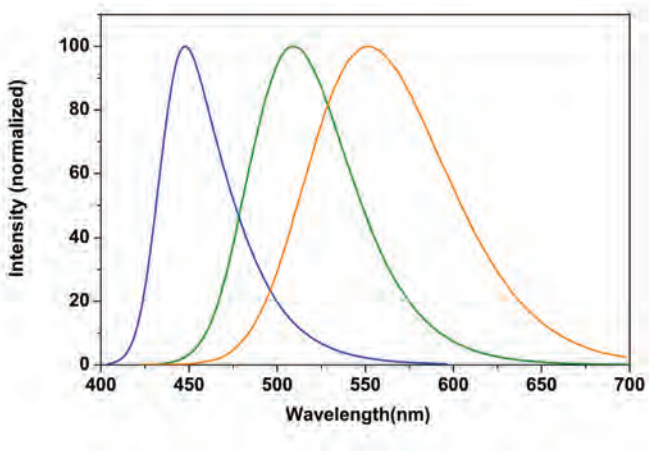

Figure 2.2. The normalized fluorescence spectra of mFluor™ Violet 450, 510 and 540 (Cat# 1150, 1151, 1152).

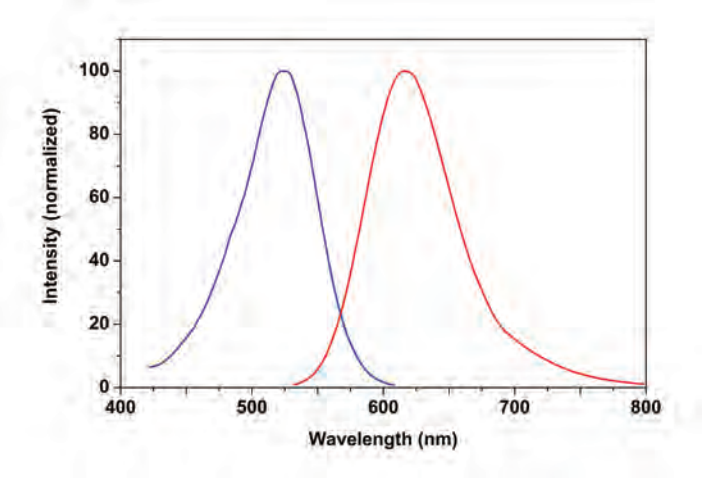

Figure 2.3. The excitation and emission spectra of mFluor™ Green 620 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

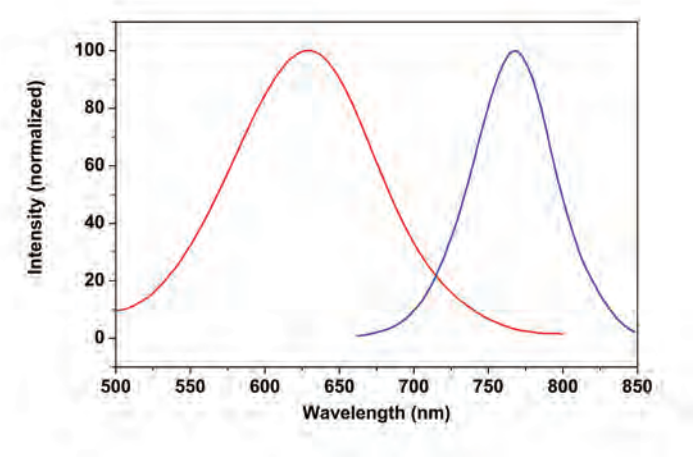

Figure 2.4. The excitation and emission spectra of mFluor™ Red 780 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

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2.3 ReadiLink™ Antibody Labeling Technology

ReadiLink™ antibody labeling technology provides a convenient way to label antibodies using a stable reactive form of the iFluor™ dyes or other fluorescent dyes (such as mFluor™ dyes). The reactive iFluor™ and mFluor™ dyes show good reactivity and selectivity with the aliphatic amines of antibodies and forms a carboxamide bond, which is identical to and is as stable as the natural peptide bond. iFluor™- and mFluor™-antibody conjugates may be used for immunofluorescent staining, fluorescent *in situ* hybridization, flow cytometry and other biological applications. Each kit comes with all the essential components for performing the conjugation reaction and for purifying the iFluor™- and mFluor™-antibody conjugates. ReadiLink™ Kits only require two simple mixing steps to produce the desired conjugates for flow cytometry and fluorescence imaging applications. The conjugation kits provide the best method for readily labeling small amount of antibodies without requiring column purification.

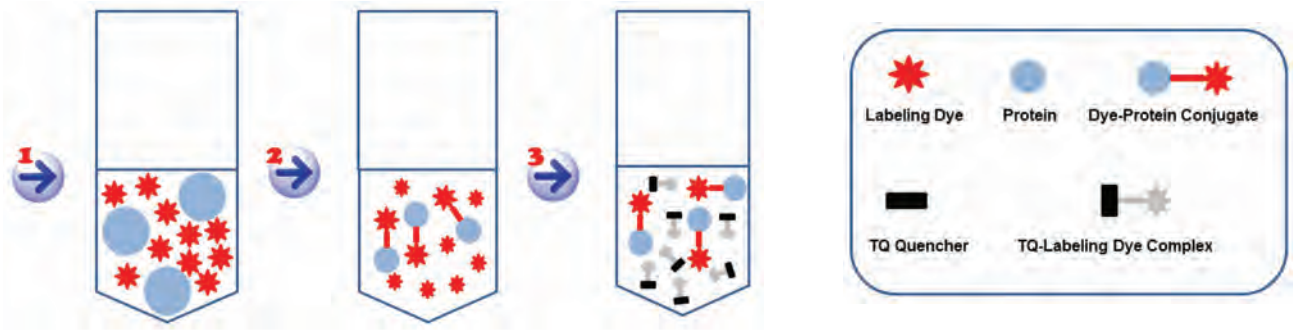

Figure 2.5. ReadiLink™ Kit Labeling Principle

- **Step 1.** Start the labeling reaction by mixing a labeling dye (red) with a protein (light blue) in a reaction buffer (pH 7.5-8.5).
- **Step 2.** Incubate the reaction solution and get a mixture of the desired protein conjugate (blue head with a red tail) and unreactive free dye (red).
- **Step 3.** Quench the reaction by mixing a non-fluorescent Tide Quencher™ (TQ, black) dye with the reaction solution. The TQ dye stops the reaction AND converts the unreactive free labeling dye to the non-fluorescent TQ-labeling dye complex (black head with a gray tail), which eliminates the background fluorescence interference of the free labeling dye.

Key Features of ReadiLink™ Kits

- *Convenient, all the components provided in the kits*
- *Robust, only two simple mixing steps required*
- *Rapid, less than 10-minute hands-on time*

Table 2.6 ReadiLink™ iFluor™ Labeling Kits

Cat. #	Product Name	Alternative to	Size	Ex (nm)	Em (nm)
1220	ReadiLink™ iFluor™ 350 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	345	442
1255	ReadiLink™ iFluor™ 488 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	491	514
1227	ReadiLink™ iFluor™ 555 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	559	569
1230	ReadiLink™ iFluor™ 594 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	592	614
1260	ReadiLink™ iFluor™ 633 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	638	655
1235	ReadiLink™ iFluor™ 647 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	654	674
1240	ReadiLink™ iFluor™ 680 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	682	701
1245	ReadiLink™ iFluor™ 700 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	693	713
1250	ReadiLink™ iFluor™ 750 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	753	779
1265	ReadiLink™ iFluor™ 790 antibody labeling kit	Lightning-Link® dye labeling kits	2 labelings	782	811

Table 2.7 ReadiLink™ mFluor™ Labeling Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
1120	ReadiLink™ mFluor™ Blue 570 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	553	570
1123	ReadiLink™ mFluor™ Green 620 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	522	617
1130	ReadiLink™ mFluor™ Red 700 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	657	700
1131	ReadiLink™ mFluor™ Red 780 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	629	780
1105	ReadiLink™ mFluor™ Violet 420 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	398	411
1100	ReadiLink™ mFluor™ Violet 450 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	403	454
1110	ReadiLink™ mFluor™ Violet 510 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	414	508
1114	ReadiLink™ mFluor™ Violet 540 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	405	537
1126	ReadiLink™ mFluor™ Yellow 630 antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	561	630

Table 2.8 Other ReadiLink™ Labeling Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
1311	ReadiLink™ APC antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	651	662
5501	ReadiLink™ BSA conjugation kit	1 kit	N/A	N/A
1299	ReadiLink™ FITC antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	492	516
5502	ReadiLink™ KLH conjugation kit	1 kit	N/A	N/A
1312	ReadiLink™ PerCP antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	482	677
5521	ReadiLink™ protein biotinylation kit *powered by ReadiView™ biotin visionization technology*	1 kit	N/A	N/A
1310	ReadiLink™ RPE antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	565	575
1300	ReadiLink™ trFluor™ Eu antibody labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	346	617
1305	ReadiLink™ trFluor™ Tb protein labeling kit *microscale optimized for labeling 50 µg antibody per reaction*	2 labelings	330	544

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2.4 Secondary Detection Reagents

Anti-IgG Secondary Antibodies

A secondary antibody is used to detect an unconjugated primary antibody that has bound to a target antigen. Secondary antibodies conjugated to enzymes and labels are key components of detection systems. Selection of an optimum secondary antibody can improve staining and reduce false positive or negative staining. AAT Bioquest's range of secondary reagents has been carefully selected to provide optimum quality and flexibility. Secondary antibodies are available in many formats and are useful in a wide range of applications, including flow cytometry (iFluor™, mFluor™, FITC and RPE), immunocytochemistry (HRP & alkaline phosphatase) and Western blotting (HRP & Biotin).

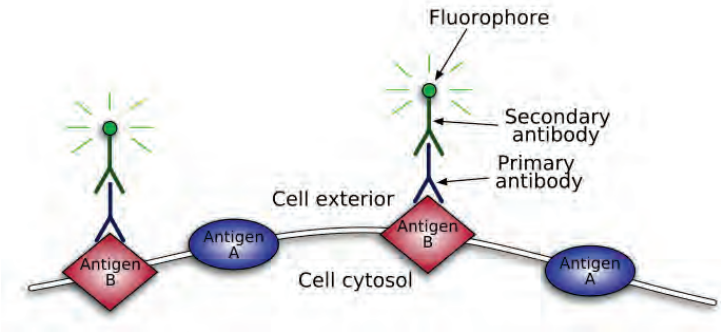

Figure 2.6. Secondary antibody action mechanism.

Cross-Adsorbed Secondary Antibodies: Some of our secondary antibodies have been adsorbed with animal or human IgG. These antibodies are designed for particular applications to reduce non-specific background staining. For example, if working with rat tissues or cells, choose a secondary antibody that has been adsorbed with rat serum or IgG. However, such adsorbed antibodies have greatly reduced epitope recognition and may recognize some subclasses of IgG very weakly, especially those subclasses which are most closely homologous to the species they were adsorbed against. For example, do not use an anti-mouse IgG that has been adsorbed against rat IgG unless you are trying to detect a mouse primary antibody in rat tissue that contains rat immunoglobulin, or in some other tissue in the presence of a rat primary antibody. Conversely, if you wish to detect a mouse primary antibody in the absence of rat immunoglobulins, it is best to use an anti-mouse secondary antibody that has not been adsorbed against rat.

Labeled Secondary Antibodies: In general, secondary antibodies can be either enzyme labeled (peroxidase and alkaline phosphatase), fluorescence labeled (FITC, iFluor™ and mFluor™) or biotin conjugated. Peroxidase is economical, rapid and more stable, while the alkaline phosphatase on the other hand is considered more sensitive than peroxidase particularly when colorimetric detection is used. Fluorescent labeled antibodies are commonly used for double or multiple staining methods.

iFluor™ Dye-Labeled Antibodies: AAT Bioquest iFluor™ dyes are optimized for labeling proteins, in particular, antibodies. These dyes are bright, photostable and have minimal quenching on proteins. They can be well excited by the major laser lines of fluorescence instruments (e.g., 350, 405, 488, 555 and 633 nm). The almost identical spectral characteristics to those of Alexa Fluor® and DyLight™ make iFluor™-labeled secondary antibody conjugates an excellent alternative to the anti-IgG conjugates of Alexa Fluor® and DyLight™.

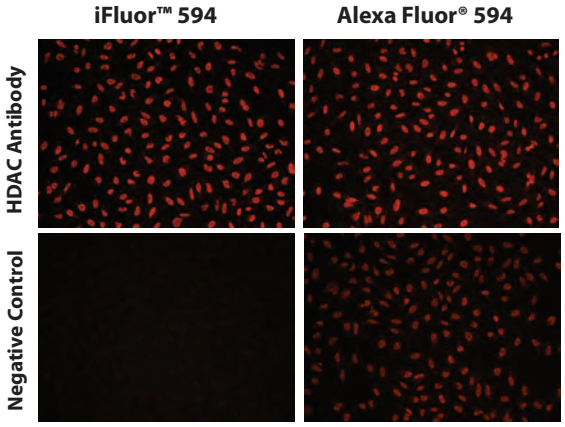

Figure 2.7. HeLa cells were stained with rabbit HDAC antibody and followed with iFluor™ 594 Goat Anti-Rabbit IgG conjugate (Cat# 16628) and Alexa Fluor® 594 Goat Anti-Rabbit IgG conjugate respectively under the same conditions. The iFluor™ 594 Goat Anti-Rabbit IgG conjugate (left panel) demonstrated much lower staining background than the corresponding Alexa Fluor® 594 (right panel).

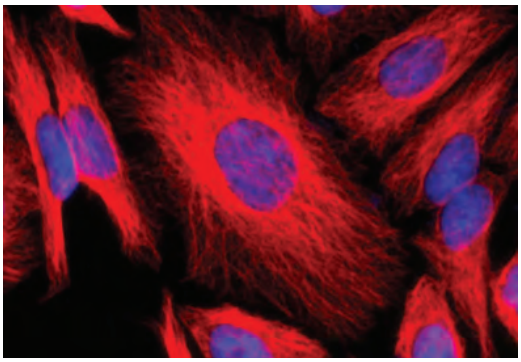

Figure 2.8. HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 594 Goat Anti-Mouse IgG (red, Cat# 16468), and nuclei were stained with Hoechst 33342 (blue, Cat# 17530).

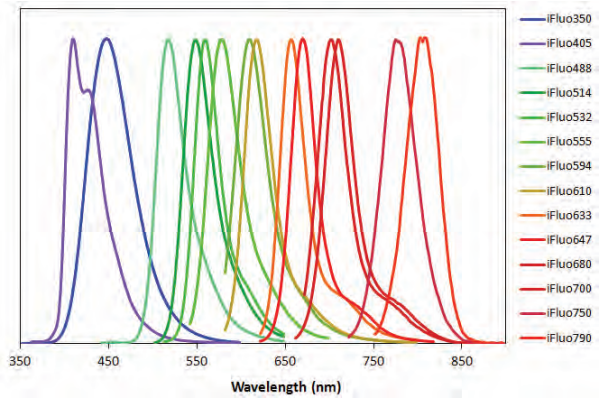

Figure 2.9. The normalized emission spectra of iFluor™ labeled secondary antibodies.

Table 2.9 iFluor™ Dye-Labeled Secondary Antibodies

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
16440	iFluor™ 350 goat anti-mouse IgG (H+L)	200 µg	345	442
16444	iFluor™ 405 goat anti-mouse IgG (H+L)	200 µg	401	420
16448	iFluor™ 488 goat anti-mouse IgG (H+L)	200 µg	491	514
16460	iFluor™ 555 goat anti-mouse IgG (H+L)	200 µg	559	569
16468	iFluor™ 594 goat anti-mouse IgG (H+L)	200 µg	592	614
16478	iFluor™ 633 goat anti-mouse IgG (H+L)	200 µg	638	655
16482	iFluor™ 647 goat anti-mouse IgG (H+L)	200 µg	654	674
16486	iFluor™ 680 goat anti-mouse IgG (H+L)	200 µg	682	701
16494	iFluor™ 700 goat anti-mouse IgG (H+L)	200 µg	693	713
16506	iFluor™ 750 goat anti-mouse IgG (H+L)	200 µg	753	779
16507	iFluor™ 790 goat anti-mouse IgG (H+L)	200 µg	782	811
16520	iFluor™ 350 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	345	442
16524	iFluor™ 405 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	401	420
16528	iFluor™ 488 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	491	514
16540	iFluor™ 555 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	559	569
16548	iFluor™ 594 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	592	614
16558	iFluor™ 633 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	638	655
16562	iFluor™ 647 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	654	674
16566	iFluor™ 680 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	682	701
16574	iFluor™ 700 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	693	713
16586	iFluor™ 750 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	753	779
16587	iFluor™ 790 goat anti-mouse IgG (H+L) *cross adsorbed*	200 µg	782	811
16600	iFluor™ 350 goat anti-rabbit IgG (H+L)	200 µg	345	442
16604	iFluor™ 405 goat anti-rabbit IgG (H+L)	200 µg	401	420
16608	iFluor™ 488 goat anti-rabbit IgG (H+L)	200 µg	491	514
16620	iFluor™ 555 goat anti-rabbit IgG (H+L)	200 µg	559	569
16628	iFluor™ 594 goat anti-rabbit IgG (H+L)	200 µg	592	614
16638	iFluor™ 633 goat anti-rabbit IgG (H+L)	200 µg	638	655
16642	iFluor™ 647 goat anti-rabbit IgG (H+L)	200 µg	654	674
16646	iFluor™ 680 goat anti-rabbit IgG (H+L)	200 µg	682	701
16652	iFluor™ 700 goat anti-rabbit IgG (H+L)	200 µg	693	713
16660	iFluor™ 750 goat anti-rabbit IgG (H+L)	200 µg	753	779
16661	iFluor™ 790 goat anti-rabbit IgG (H+L)	200 µg	782	811
16670	iFluor™ 350 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	345	442
16674	iFluor™ 405 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	401	420
16678	iFluor™ 488 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	491	514
16690	iFluor™ 555 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	559	569
16698	iFluor™ 594 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	592	614
16704	iFluor™ 633 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	638	655
16710	iFluor™ 647 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	654	674
16712	iFluor™ 680 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	682	701
16714	iFluor™ 700 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	693	713
16720	iFluor™ 750 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	753	779
16721	iFluor™ 790 goat anti-rabbit IgG (H+L) *cross adsorbed*	200 µg	782	811

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trFluor™ Dye-Labeled Antibodies: Many biological compounds present in cells, serum or other biological fluids are naturally fluorescent, and thus the use of conventional, prompt fluorophores leads to serious limitations in assay sensitivity due to the high background caused by the autofluorescence of the biological molecules to be assayed. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interferences. Our trFluor™ probes enable time-resolved fluorometry (TRF) for the assays that require high sensitivity. trFluor™ probes have large Stokes shifts and extremely long emission half-lives when compared to more traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to the other TRF compounds, our trFluor™ probes have relatively high stability, high emission yield and the ability to be linked to biomolecules. The trFluor™ anti-mouse IgG (H+L) conjugates are commonly used as second step reagents.

Key Features of trFluor™ Dyes

- *No fluoride addition is required*
- *No enhancing solution is required*
- *Available in a variety of reactive forms*
- *Much easier to be conjugated to biomolecules*
- *Much higher conjugation yield than other TRF dyes*
- *Maximally excited by the common light sources at ~350 nm*
- *trFluor™ Eu dye is optimized to pair with APC, iFluor™ 647, TF5, Cy5®, DyLight™ 650 and Alexa Fluor® 647*
- *trFluor™ Tb dye is optimized to pair with FITC, iFluor™ 488, TF2, DyLight™ 488 and Alexa Fluor® 488*

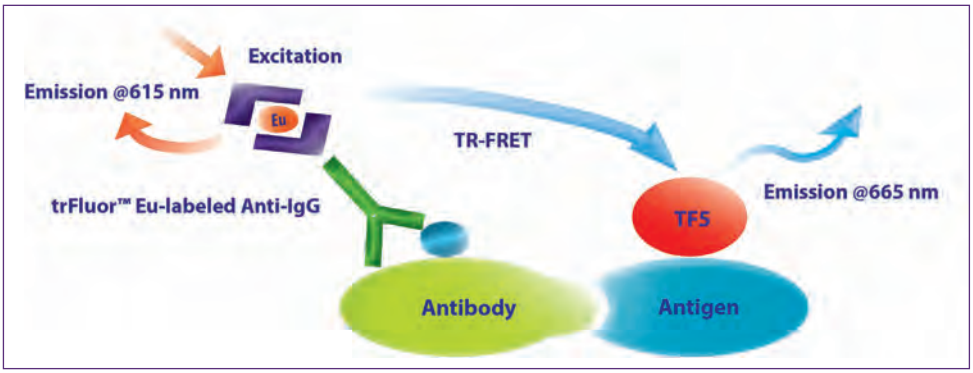

Figure 2.10. TR-FRET assay principle using trFluor™ Eu as the donor and Tide Fluor™ 5 (TF5) as the acceptor.

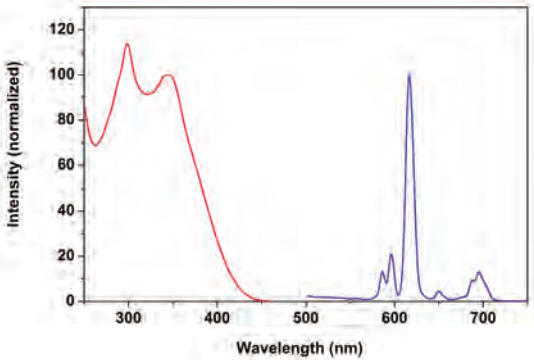

Figure 2.11. The excitation and emission spectra of trFluor™ Eu Goat Anti-Rabbit IgG conjugate (Cat# 16668) in PBS buffer (pH 7.2).

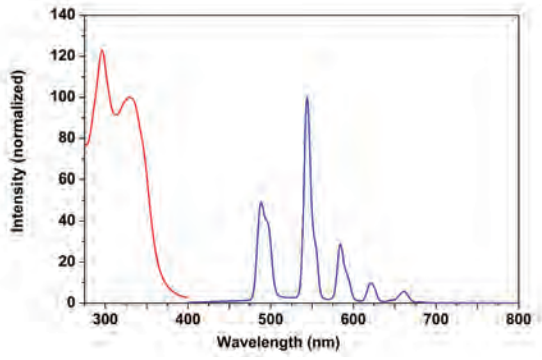

Figure 2.12. The excitation and emission spectra of trFluor™ Tb Goat Anti-Rabbit IgG conjugate (Cat# 16669) in PBS buffer (pH 7.2).

Table 2.10 trFluor™ Dye-Labeled Antibodies

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
16518	trFluor™ Eu goat anti-mouse IgG (H+L)	100 µg	346	617
16668	trFluor™ Eu goat anti-rabbit IgG (H+L)	100 µg	346	617
16725	trFluor™ Eu goat anti-rabbit IgG (H+L) *cross adsorbed*	100 µg	346	617
16519	trFluor™ Tb goat anti-mouse IgG (H+L)	100 µg	330	544
16599	trFluor™ Tb goat anti-mouse IgG (H+L) *cross adsorbed*	100 µg	330	544
16669	trFluor™ Tb goat anti-rabbit IgG (H+L)	100 µg	330	544
16726	trFluor™ Tb goat anti-rabbit IgG (H+L) *cross adsorbed*	100 µg	330	544

Streptavidin Conjugates

The avidin/streptavidin-biotin interaction is the strongest known non-covalent biological interaction (K_d=10^{-15} M) between a protein and its ligand. One avidin binds four biotins as shown in Figure 2.13. The bond formation between biotin and avidin/streptavidin is very rapid and, once formed, is unaffected by pH, organic solvents and other denaturing agents. Both avidin and streptavidin have essentially irreversible biotin-binding properties since bound biotin can only be released by denaturing the subunits of the proteins. The tight and specific binding of biotin and its derivatives to various avidins has been extensively explored for a number of biological applications.

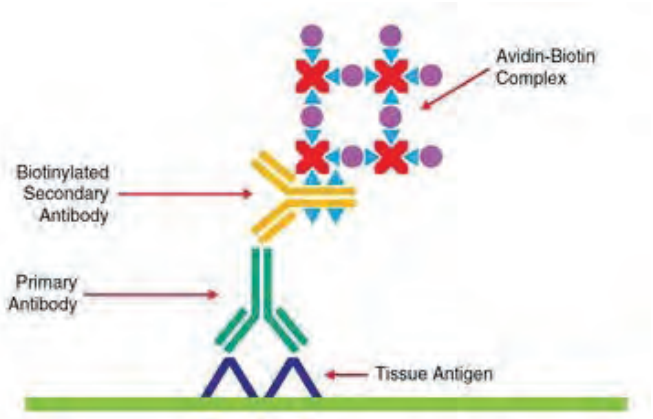

Figure 2.13. The streptavidin-based assay principle.

iFluor™ Dye-Labeled Streptavidins: AAT Bioquest iFluor™ dyes are optimized for labeling proteins. These dyes are bright, photostable and have minimal quenching on proteins. They can be well excited by the major laser lines of fluorescence instruments (e.g., 350, 405, 488, 555 and 633 nm). The almost identical spectral characteristics make iFluor™-labeled streptavidin conjugates an excellent alternative to the streptavidin conjugates of Alexa Fluor® and DyLight™.

Table 2.11 iFluor™ Dye-Labeled Streptavidins

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
16950	iFluor™ 350-streptavidin conjugate	200 µg	345	442
16952	iFluor™ 405-streptavidin conjugate	200 µg	401	420
16955	iFluor™ 488-streptavidin conjugate	200 µg	491	514
16956	iFluor™ 514-streptavidin conjugate	200 µg	518	542
16957	iFluor™ 532-streptavidin conjugate	200 µg	542	558
16959	iFluor™ 555-streptavidin conjugate	200 µg	559	569
16962	iFluor™ 594-streptavidin conjugate	200 µg	592	614
16965	iFluor™ 633-streptavidin conjugate	200 µg	638	655
16966	iFluor™ 647-streptavidin conjugate	200 µg	654	674
16968	iFluor™ 680-streptavidin conjugate	200 µg	682	701
16970	iFluor™ 700-streptavidin conjugate	200 µg	693	713
16973	iFluor™ 750-streptavidin conjugate	200 µg	753	779

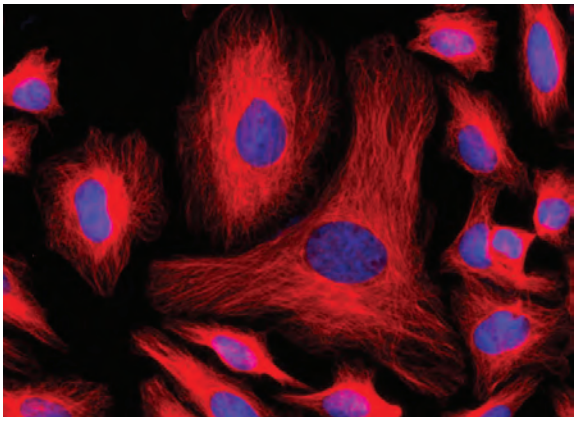

Figure 2.14. Image of tubulins in HeLa cells. Tubulins were stained using mouse anti-α-tubulin antibody followed with biotinylated Goat Anti-Mouse IgG staining, and visualized with red fluorescent iFluor™555-streptavidin conjugate (Cat# 16959). Nuclei were stained with blue fluorescent Hoechst 33342 (Cat# 17535).

mFluor™ Dye-Labeled Streptavidins: AAT Bioquest mFluor™ dyes are developed for multicolor flow cytometry-focused applications. These dyes have large Stokes Shifts, and can be well excited by the laser lines of flow cytometers (e.g., 405 nm, 488 nm and 633 nm). Our mFluor™ bioconjugates are excellent replacements to those of Pacific Blue™, Pacific Green™ and Pacific Orange™ due to their superior performance. Some of our mFluor™ conjugates are alternatives to the phycobiliprotein-dye tandems that are difficult to make.

Table 2.12 mFluor™ Dye-Labeled Streptavidins

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
16935	mFluor™ Blue 570-streptavidin conjugate	100 µg	553	570
16938	mFluor™ Green 620-streptavidin conjugate	100 µg	523	617
16946	mFluor™ Red 700-streptavidin conjugate	100 µg	657	700
16948	mFluor™ Red 780-streptavidin conjugate	100 µg	629	780
16930	mFluor™ Violet 450-streptavidin conjugate	100 µg	403	454
16931	mFluor™ Violet 510-streptavidin conjugate	100 µg	414	508
16932	mFluor™ Violet 540-streptavidin conjugate	100 µg	405	537
16942	mFluor™ Yellow 630-streptavidin conjugate	100 µg	611	630

Phycobiliprotein-Labeled Streptavidins: AAT Bioquest's phycobiliprotein-streptavdin conjugates are optimized for multicolor flow cytometry applications. They can also be used for other multiplexing applications, e.g., with a variety of Luminex® bioanalytical platforms. The phycobiliproteins are composed of a number of subunits, each having a protein backbone to which linear tetrapyrrole chromophores are covalently bound. Phycoerythrins (red) and phycocyanins (blue) are the two major classes of phycobiliproteins. The absorption maxima for phycoerythrins (PE) lie between 490 and 570 nm while absorption maxima for phycocyanins (PC) are found between 610 and 665 nm. In general, phycobiliproteins have good long-term stability when stored refrigerated as ammonium sulfate precipitates. Phycobiliproteins are much more sensitive than the small organic dyes.

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Table 2.13 Phycobiliprotein-Labeled Streptavidins

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
16908	APC-iFluor™ 750-streptavidin conjugate	100 µg	651	779
16902	APC-streptavidin conjugate	100 µg	651	662
16905	PerCP-streptavidin conjugate	100 µg	482	667
16906	RPE-iFluor™ 647-streptavidin conjugate	100 µg	565	674
16907	RPE-iFluor™ 750-streptavidin conjugate	100 µg	565	779
16900	RPE-streptavidin conjugate	100 µg	565	575
16901	RPE-streptavidin conjugate	1 mg	565	575

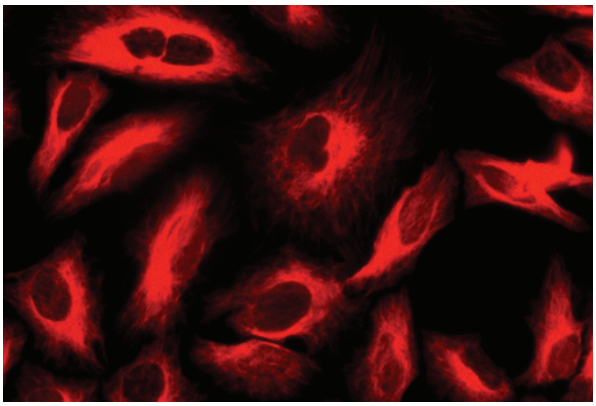

Figure 2.15. Image of tubulins in HeLa cells. Tubulins were stained using mouse anti-α-tubulin antibody followed with biotinylated Goat Anti-Mouse IgG staining, and visualized with red fluorescent RPE-streptavidin conjugate (Cat# 16901).

trFluor™ Dye-Labeled Streptavidins: Many biological compounds present in cells, serum or other biological fluids are naturally fluorescent, and thus the use of conventional, prompt fluorophores leads to serious limitations in assay sensitivity due to the high background caused by the autofluorescence of the biological molecules to be assayed. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interferences. Our trFluor™ probes enable time-resolved fluorometry (TRF) for the assays that require high sensitivity. trFluor™ probes have large Stokes shifts and extremely long emission half-lives when compared to more traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to the other TRF compounds, our trFluor™ probes have relatively high stability, high emission yield and ability to be linked to biomolecules. trFluor™ -streptavidin conjugate comprises streptavidin (as the biotin-binding protein) with trFluor™ dye covalently attached (as the time-resolved red fluorescent europium label). It is commonly used as a second step reagent for indirect immunofluorescent staining, when used in conjunction with biotinylated primary antibodies. It is a very valuable tool for biotin-streptavidin-based biological assays and tests using TR-FRET platform. A variety of the complementary biotinylated reagents are available from numerous commercial vendors.

Table 2.14 trFluor™ Dye-Labeled Streptavidins

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
16925	trFluor™ Eu-streptavidin conjugate	100 µg	346	617
16926	trFluor™ Tb-streptavidin conjugate	100 µg	330	544

Enzyme-Labeled Streptavidins: Streptavidin conjugates are widely used together with a conjugate of biotin for specific detection of a variety of proteins, protein motifs, nucleic acids and other molecules since streptavidin has a very high binding affinity for biotin. The enzyme-streptavidin conjugate comprises streptavidin (as the biotin-binding protein) with an enzyme covalently attached (as the enzyme label). It is commonly used as a second step reagent for indirect immunofluorescent staining, when used in conjunction with an enzyme substrate (such as our sensitive fluorogenic ADHP substrate for HRP-streptavidin conjugate). It is a very valuable tool for biotin-streptavidin-based biological assays and tests. A variety of the complementary biotinylated reagents are available from numerous commercial vendors.

Table 2.15 Enzyme-Labeled Streptavidins

Cat. #	Product Name	Size
16921	AP-streptavidin conjugate	1 mg
16920	HRP-streptavidin conjugate	1 mg

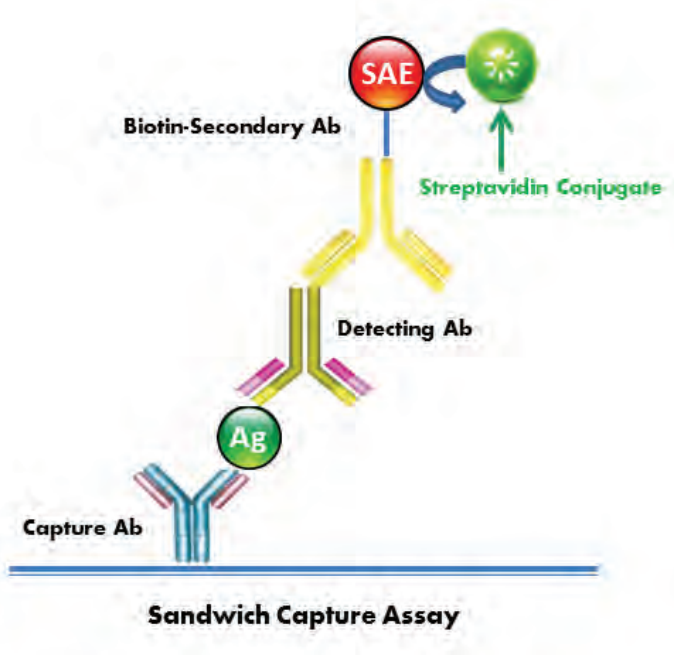

Figure 2.16. The sandwich ELISA assay principle. Enzyme- or fluorescence- labeled streptavidin conjugates (SAE) are used as the detection tags.

FluoroQuest™ Anti-Fading Reagents

The photon output of a dye represents the average number of cycles of excitation followed by fluorescence emission that the dye goes through before it is irreversibly photobleached. The average photon output is defined by the ratio of fluorescence quantum efficiency to photobleaching quantum efficiency. When exposed to excitation light, fluorescence intensity of dyes decreases due to their photooxidation or other photoreactions. It is ideal to have the maximal ratio of fluorescence quantum efficiency to photobleaching quantum efficiency. However, very few fluorescent organic dyes can completely resist photobleaching. Frequently, when a section has been scanned repeatedly under strong excitation light, dyes could lose significant fluorescence signal before visual evaluation or photography can be accomplished. For example, the photobleaching of fluoresceins (such as FITC-labeled antibodies) has become a major problem in fluorescence microscopy. In severe cases (such as phycoprotein-labeled bioconjugates), a fluorescence image with high resolution cannot even be taken due to the extremely high photobleaching rate.

The main purpose of FluoroQuest™ Anti-Fading Kits is to reduce the dye photobleaching rate, giving researchers longer observation time. The kits are recommended for fixed cells and tissues that have been stained with fluorescent dyes or their biological conjugates (such as dye-antibody conjugates). Cells can be viewed immediately. If long term storage is desired, the reagent can be allowed to harden overnight. However, the staining patterns of FluoroQuest™ Anti-Fading Kits are sharper if viewed immediately. Our fluorescent reagents and assay kits have been extensively benchmarked for live cell analysis applications and are optimal for demanding cell analysis applications involving confocal microscopy, flow cytometry, microplate readers and HCS/HTS, where consistency and reproducibility are required.

FluoroQuest™ Anti-Fading Kit I contains 3 sampler components for different imaging experiments. The components are all premixed and ready-to-use solutions. To be complementary to Kit I (the slide format), our FluoroQuest™ Anti-Fading Kit II is optimized for the microplate format. Unlike Kit I, Kit II provides only one formulation specifically optimized for FITC based experiments.

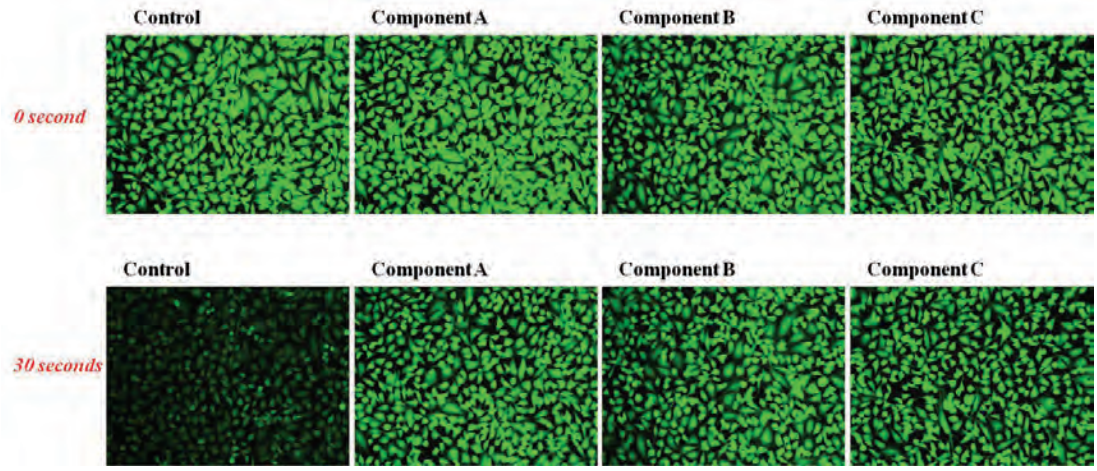

Figure 2.17. U2OS cells in a 96-well Costar black wall/clear bottom plate were loaded with 1 µM calcein, AM for 1 hour and fixed with 4% formaldehyde for 30 minutes. FluoroQuest™ Anti-Fading Kit 1 (Cat# 20001) were added to the samples after removing all the media. The FITC signals were compared at 0 and after 30 seconds exposure using an Olympus fluorescence microscope. The same exposure settings were used for all the images.

Table 2.16 Fluorescence Imaging and Flow Cytometry Accessory Reagents

Cat. #	Product Name	Size
20001	FluoroQuest™ anti-fading kit I	1 kit
20003	FluoroQuest™ anti-fading kit II	1 kit
20006	FluoroQuest™ fluorescence signal enhancing solution	5 mL
20004	FluoroQuest™ mounting medium with DAPI	50 mL
20053	Pluronic® F-127 *10% solution in water*	10 mL
20052	Pluronic® F-127 *20% solution in DMSO*	10 mL
20060	Probenecid *cell culture tested*	10x72 mg
20010	ReadiUse™ 4% formaldehyde fixation solution	50 mL
20012	ReadiUse™ mammalian cell lysis buffer *5X*	10 mL
20009	ReadiUse™ microscope mounting solution	50 mL
20061	ReadiUse™ probenecid, sodium salt *water-soluble*	10x77 mg
20062	ReadiUse™ probenecid *25 mM stabilized aqueous solution*	10x10 mL

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Cell Labeling and Tracking Probes

3

cell labeling and tracking probes at-a-glance*

	Fluorescence Imaging	Flow Cytometry
Labeling Dead Cells		22500, 22501, 22502, 22600, 22601, 22602, 22603, 22604
Labeling Live Cells	22606, 22607, 22609, 22614, 22615, 22616	22606, 22607, 22609, 22614, 22615, 22616
Monitoring Cell Proliferation		22022, 22028, 22251, 22252, 22253, 22254, 22255, 22256, 22257, 22258, 22022, 22028
Tracking Cell Cycles		22841, 22842
Tracking Live Cells	22620, 22621, 22622, 22623, 22624	22620, 22621, 22622, 22623, 22624

* Products listed by catalog number

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Cell Labeling and Tracking Probes

3.1 Live Cell Labeling Probes

Properly labeling cells is one of the essential tasks for analyzing cells. Cell Explorer™ fluorescence labeling kits are a set of tools which can be used to label cells for fluorescence microscopic and flow cytometric investigations of cellular functions. The effective labeling of cells provides a powerful method for studying cellular events in a spatial and temporal context.

Cell Explorer™ Live Cell Labeling Kits are designed to uniformly label live cells with a proprietary dye whose fluorescence is strongly enhanced upon entering into live cells. The dye is a hydrophobic compound that easily permeates intact live cells. The hydrolysis of the weakly fluorescent substrate by intracellular esterases generates a strongly fluorescent hydrophilic product that is well-retained in the cell cytoplasm. Cells grown on black wall/clear bottom plates or slides can be stained and quantified in less than two hours. The kits can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope. They are useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity.

Our Cell Explorer™ Live Cell Labeling Kits provide all the essential components with an optimized cell-labeling protocol, and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells). A full set of different fluorescent dyes are used with our kits, providing an excellent tool set for multicolor cell analysis applications (see Figure 3.2). For example, kits 22614, 22615 and 22616 are optimized to use with a flow cytometer equipped with 405 nm violet laser.

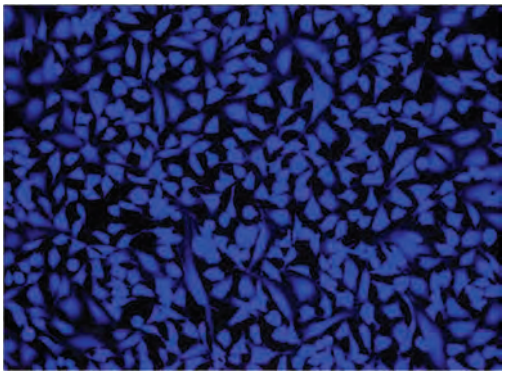

Figure 3.1. Image of HeLa cells stained with Cell Explorer™ Live Cell Labeling Kit (Cat#

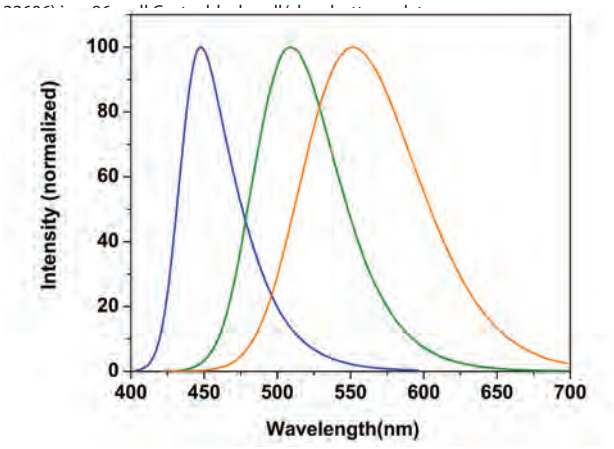

Figure 3.2. The emission spectra of the live cell labeling probes (Ex = 405 nm) used in Cell Explorer™ Live Cell Labeling Kits (Blue: Cat# 22614; Green: Cat# 22615; Orange: Cat# 22616).

Table 3.1 Live Cell Labeling Probes and Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22004	Calcein, AM *UltraPure grade*	20x50 µg	495	515
22007	Calcein blue, AM	1 mg	360	445
22009	Calcein Orange™	1 mg	525	550
22010	Calcein Red™	1 mg	646	659
22606	Cell Explorer™ live cell labeling kit *blue fluorescence*	1 kit	360	445
22614	Cell Explorer™ live cell labeling kit *blue fluorescence with 405 nm excitation*	1 kit	410	450
22607	Cell Explorer™ live cell labeling kit *green Fluorescence*	1 kit	495	515
22615	Cell Explorer™ live cell labeling kit *green fluorescence with 405 nm excitation*	1 kit	410	500
22616	Cell Explorer™ live cell labeling kit *orange fluorescence with 405 nm excitation*	1 kit	398	545
22609	Cell Explorer™ live cell labeling kit *red fluorescence*	1 kit	646	659
22012	CytoCalcein™ Violet 450 *excited at 405 nm*	1 mg	408	450
22013	CytoCalcein™ Violet 500 *excited at 405 nm*	1 mg	410	500
17540	Nuclear Green™ LCS1	0.5 mL	503	526
17541	Nuclear Orange™ LCS1	0.5 mL	514	555
17542	Nuclear Red™ LCS1	0.5 mL	622	645

3.2 Live Cell Tracking Kits

The effective labeling of cells offers a powerful method for studying cellular events in a spatial and temporal context. Labeling cells with a fluorescent tag that very well stays in cells provides an excellent tool for monitoring cells. Our Cell Explorer™ Live Cell Tracking Kits use a set of proprietary fluorescent tracking dyes that get enhanced fluorescence upon entering into live cells. The dyes used in the kits are hydrophobic compounds that easily permeate intact live cells. The hydrolysis of the weakly fluorescent substrates by intracellular esterases generates strongly fluorescent hydrophilic products that are well-retained in the cell cytoplasm. Our cell tracking dyes have good photostability with robust imaging performance.

Cell Explorer™ live cell tracking kits provide an effective tool of labeling cells for flow cytometric and fluorescence microscopic investigations of cellular functions. The kits are particularly suitable for multicolor flow cytometric analysis of cells. They can be used with all the common filter sets of fluorescence microscope and flow cytometers. For example, kits 22620, 22621, 22622, 22623 and 22624 are compatible with the common filter sets of AMCA, FITC, TRITC, Texas Red® and Cy5® respectively.

Our live cell tracking kits are useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. They are suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. The tracking kits provide all the essential components with an optimized cell-labeling protocol.

Key Features of Live Cell Tracking Kits

- *A full spectrum of colors available for multiplexing*
- *Compatible with either flow cytometry or fluorescence imaging*
- *Robust performance for tracking cells*
- *Minimal hands-on time required*

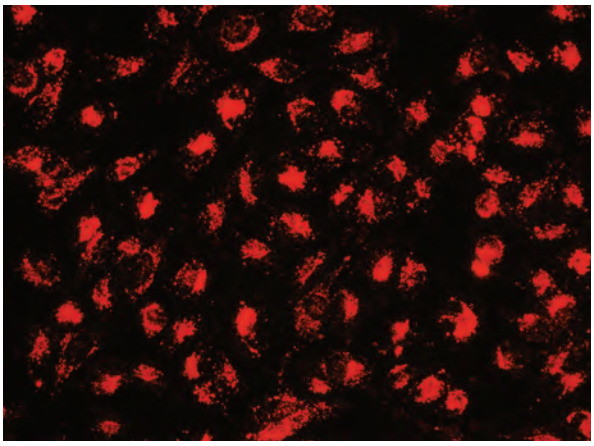

Figure 3.3. Image of HeLa cells stained with Cell Explorer™ Live Cell Tracking Kit (Cat# 22623) in a 96-well Costar black wall/clear bottom plate.

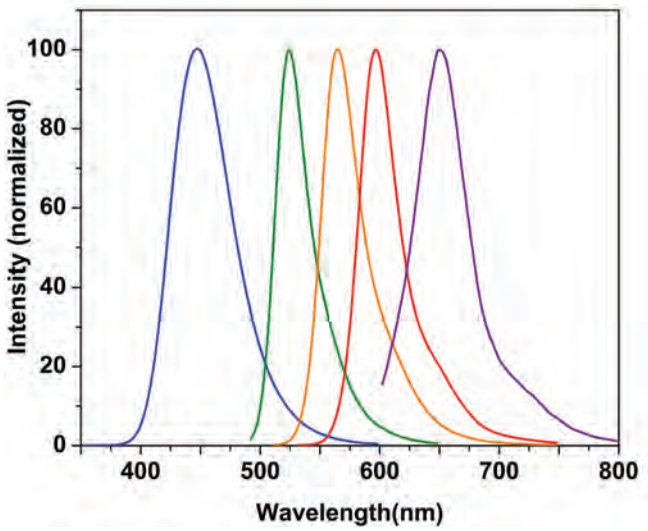

Figure 3.4. The emission spectra of live cell tracking probes used in Cell Explorer™ Live Cell Tracking Kits (Blue: Cat# 22620; Green: Cat# 22621; Orange: Cat# 22622; Red: Cat# 22623; Deep Red: Cat# 22624).

Table 3.2 Live Cell Tracking Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22620	Cell Explorer™ live cell tracking kit *blue fluorescence*	200 tests	360	445
22624	Cell Explorer™ live cell tracking kit *deep red fluorescence*	200 tests	637	650
22621	Cell Explorer™ live cell tracking kit *green fluorescence*	200 tests	495	515
22622	Cell Explorer™ live cell tracking kit *orange fluorescence*	200 tests	528	541
22623	Cell Explorer™ live cell tracking kit *red fluorescence*	200 tests	575	600

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3.3 Fixable Dead Cell Staining Kits

The effective labeling of cells provides a powerful method for studying cellular events in a spatial and temporal context. AAT Bioquest Cell Explorer™ Fixable Dead Cell Staining kits are a set of tools used to label cells for fluorescence microscopic and flow cytometric investigations of cellular functions.

Cell Explorer™ Fixable Dead Cell Staining Kits employ cell components-reactive fluorescent stains to evaluate mammalian cell viability using flow cytometry and fluorescence microscope. In cells with compromised membranes, the stains react with cell components both in the cell interior and on the cell surface, yielding intense fluorescent staining. The proprietary stains used in the kits become more fluorescent upon binding to cellular components. These cell stains are not live cell-permeable. In viable cells, the stain's reactivity is restricted to the cell-surface components, resulting in less intense fluorescence. The difference in intensity between the live and dead cell populations is quite large, and the fluorescence intensity discrimination is completely preserved following formaldehyde fixation. Moreover, these stains use only one channel of a flow cytometer, making them compatible with multiparameter staining experiments for multiplexing applications.

The fluorescence signals of the stains used in the kits are pH-independent and quite photostable. The stains have much better water solubility, making the kits easier to use. Cell Explorer™ Fixable Dead Cell Staining Kits provide all the essential components with an optimized fixable dead cell staining protocol that requires minimal hands-on time.

Key Features of Fixable Dead Cell Staining Kits

- *A full spectrum of colors available for multiplexing*
- *Compatible with either flow cytometry or fluorescence imaging*
- *Minimal hands-on time required*
- *Robust performance for tracking cells*

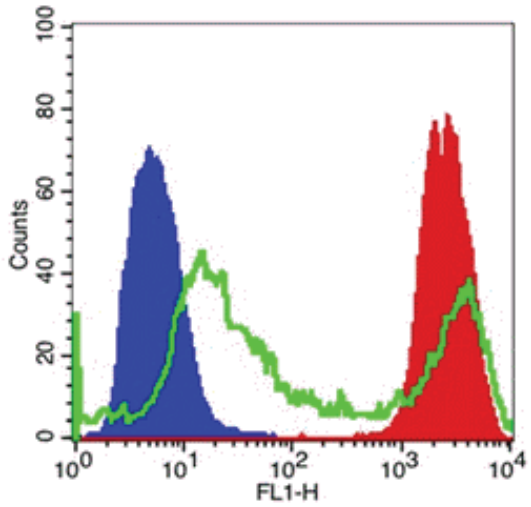

Figure 3.5. Detection of Jurkat cell viability using Cell Explorer™ Fixable Dead Cell Staining Kit (Cat# 22601). Jurkat cells were treated and stained with Stain IT™ Green and then fixed with 3.7% formaldehyde and analyzed by flow cytometry. Live (blue solid peak), staurosporine treated (green line) and heat-treated (red solid peak)cells were distinguished with Ex/Em = 488/520 nm (FL1) channel. Nearly identical results were obtained before fixation.

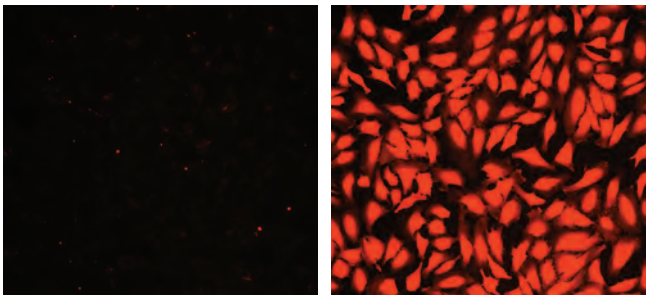

Figure 3.6. Images of HeLa cells stained with Cell Explorer™ Fixable Dead Cell Staining Kit (Cat# 22603) in a 96-well Costar black wall/clear bottom plate. Left: Live HeLa cells; Right: Fixed HeLa cells.

Table 3.3 Fixable Dead Cell Staining Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22600	Cell Explorer™ fixable dead cell staining kit *blue fluorescence*	1 kit	353	442
22500	Cell Explorer™ fixable dead cell staining kit *blue fluorescence with 405 nm excitation*	1 kit	410	450
22604	Cell Explorer™ fixable dead cell staining kit *deep red fluorescence*	1 kit	649	660
22601	Cell Explorer™ fixable dead cell staining kit *green fluorescence*	1 kit	498	521
22501	Cell Explorer™ fixable dead cell staining kit *green fluorescence with 405 nm excitation*	1 kit	408	512
22602	Cell Explorer™ fixable dead cell staining kit *orange fluorescence*	1 kit	547	573
22502	Cell Explorer™ fixable dead cell staining kit *orange fluorescence with 405 nm excitation*	1 kit	398	550
22603	Cell Explorer™ fixable dead cell staining kit *red fluorescence*	1 kit	583	603

3.4 Cell Proliferation Probes

CytoTell™ Green

Flow cytometry combined with fluorescence staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes, CFSE is the preferred cell proliferation indicator that is widely used for live cell analysis. However, there are a few severe problems associated with the use of CFSE for monitoring cell proliferation. 1). CFSE is highly toxic to cells since CFSE indiscriminately reacts with all amino groups, thus affects many critical intracellular protein functions (such as cell membrane GPCRs); 2). CFSE has slow response and is inconvenient to use. The CFSE fluorescence intensity of the 2nd generation cells is decreased more than 10 fold from the 1st generation. You would have to wait for another generation to start the cell proliferation analysis; 3). Medium removal is required. You would have to remove medium for cell analysis with a flow cytometer since CFSE reacts with medium components.

CytoTell™ Green is developed to eliminate the above CFSE limitations. CytoTell™ Green can also be used for long term tracking of labeled cells. Analysis using two-parameter plots may provide better resolution of each generation, especially between undivided cells and the first generation. Cells labeled with CytoTell™ Green may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers. CytoTell™ Green can be excited by the 488 nm blue laser line with the peak emission at 520 nm, which makes it compatible with the FITC filter set.

Key Features of CytoTell™ Green

- *Spectrally similar to CFSE and FITC*
- *Much faster response to cell proliferation than CFSE*
- *More convenient to use than CFSE*
- *More sensitive than CFSE*
- *Much more stable than CFSE*

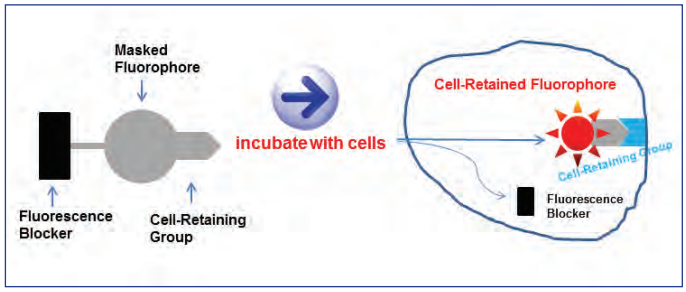

Figure 3.7. CytoTell™ dye working principle. CytoTell™ dye consists of three components: a). fluorescence blocker; b). masked fluorophore; and c). cell-retaining moiety. Upon entering live cells, the fluorescence of CytoTell™ dye is released via the removal of fluorescence blocker, and the released fluorophore is retained in cells through the cell-retaining group.

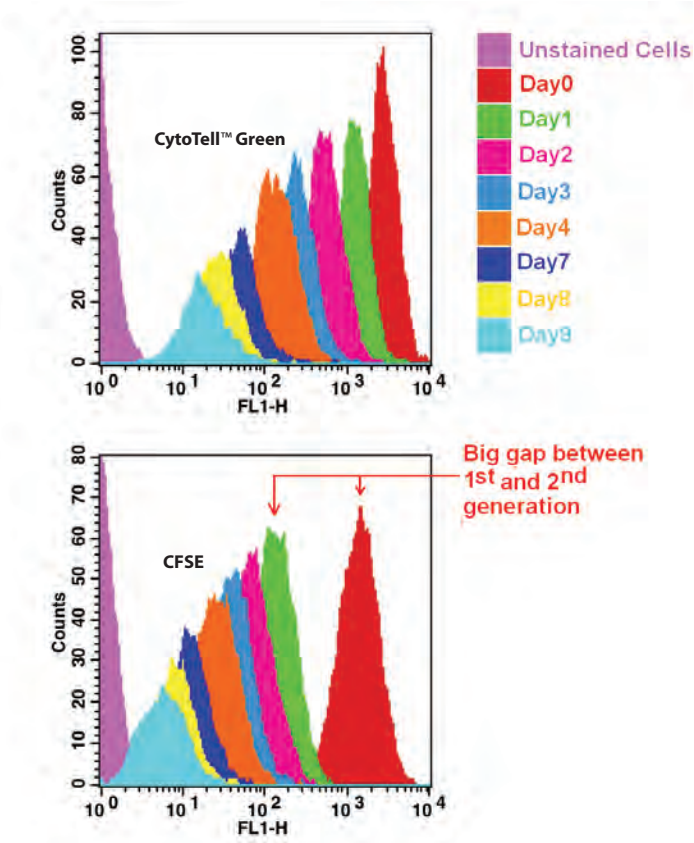

Figure 3.8. Cell tracking assays using CytoTell™ Green (Cat# 22253) and CFSE (Cat# 22022). Jurkat cells (~2x10^6 cells/mL) were stained with CytoTell™ Green or CFSE (0.5 µM) on Day 0. The cells were passed serially at 1:1 ratio for 9 days. Fluorescence intensity was measured with FACSCalibur™ flow cytometer (BD, San Jose, CA) using FL1 channel on the day after passage. Successive generations were represented by different colors.

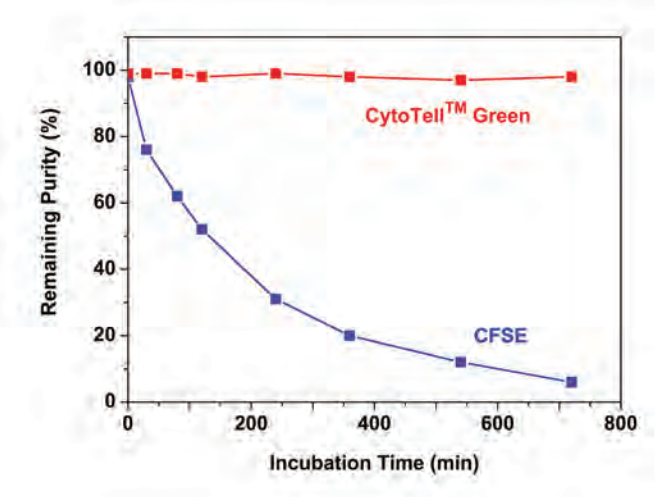

Figure 3.9. Stability comparison of CytoTell™ Green (Cat# 22253) and CFSE (Cat# 22022). 5 mM PBS working solutions of CytoTell™ Green and CFSE were monitored using HPLC (pH 7.2).

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CytoTell™ Blue

Flow cytometry combined with fluorescence staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes, CFSE is the preferred cell proliferation indicator that is widely used for live cell analysis. However, it is impossible to use CFSE and its fluorescein analogs for GFP-transfected cells or for the applications where a FITC-labeled antibody is used since CFSE and its fluorescein analogs have the excitation and emission spectra almost identical to those of GFP or FITC. CytoTell™ dyes are well excited with major laser lines such as 405 nm, 488 nm or 633 nm laser line with multicolor emissions. They have minimal cytotoxicity and are used for the multicolor applications with either GFP cell lines or FITC-labeled antibodies since they have either excitation or emission spectra distinct from those of fluorescein. CytoTell™ Blue is a blue fluorescent dye that stains cells evenly. It has a peak excitation of 405 nm and can be excited by the 405 nm violet laser line. Its peak emission of 450 nm can be detected with a 450/20 nm band pass filter (equivalent to Pacific Blue® or BD Horizon® V450), making it compatible with applications that use GFP or FITC antibodies for multicolor cell analysis.

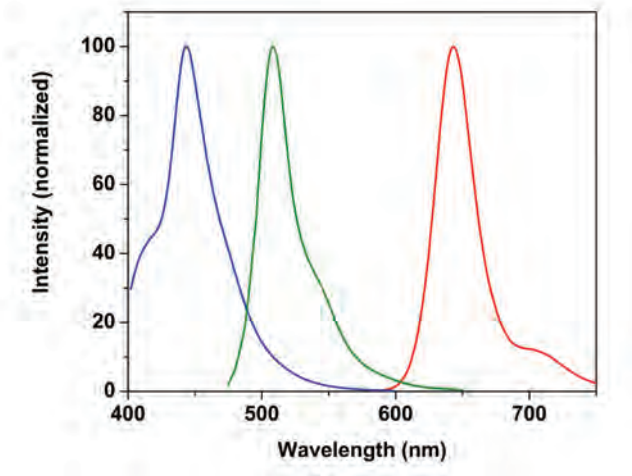

Figure 3.10. Emission spectral comparison of CytoTell™ Blue (Ex/Em = 403/454 nm, Cat# 22251), CytoTell™ Green (Ex/Em = 511/525 nm, Cat# 22253), and CytoTell™ Red (Ex/Em = 628/643 nm, Cat# 22255) in PBS buffer (pH 7.2).

CytoTell™ Red

CytoTell™ Red is a red fluorescent dye that stains cells evenly. As cells divide, the dye is distributed equally between daughter cells that can be measured as successive halving of the fluorescence intensity of the dye. Up to 8 generations of cells may be visualized using CytoTell™ Red. CytoTell™ Red can also be used for the long term tracking of labeled cells. CytoTell™ Red has a peak excitation of 630 nm and can be well excited by the 633 nm red laser line. It has a peak emission of 660 nm and can be detected with a 660/20 nm band pass filter (equivalent to APC, Alexa Fluor® 647 or Cy5®), making it compatible with the applications that use GFP or FITC antibodies for multicolor cell analysis.

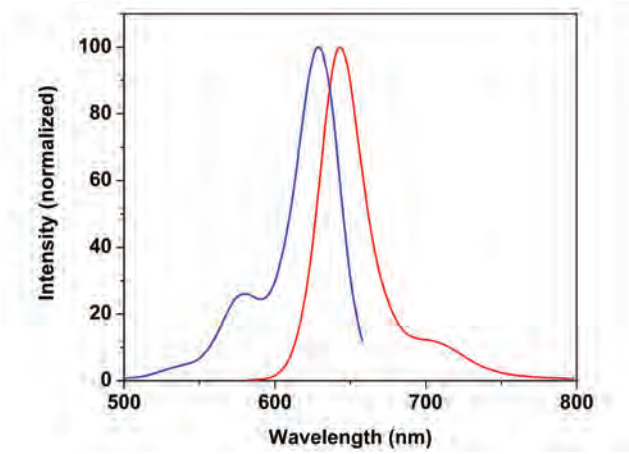

Figure 3.11. The excitation and emission spectra of CytoTell™ Red (Cat# 22255) in PBS buffer (pH 7.2).

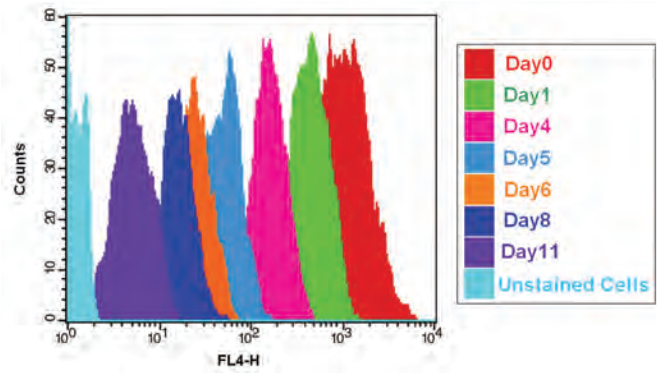

Figure 3.12. Cell tracking assay using CytoTell™ Red (Cat# 22255). Jurkat cells (~ $2x10^6$ cells/mL) were stained with CytoTell™ Red (2 µM) on Day 0. The cells were passed serially at 1:1 ratio for 11 days. Fluorescence intensity was measured with FACSCalibur™ flow cytometer (BD, San Jose, CA) in FL4 channel on the day after passage. Successive generations were represented by different colors.

Table 3.4 Cell Proliferation Probes

Cat. #	Product Name	Size
22022	CFSE [5-(and 6)-carboxyfluorescein diacetate, succinimidyl ester] *mixed isomers*	25 mg
22251	CytoTell™ Blue	500 tests
22253	CytoTell™ Green	500 tests
22257	CytoTell™ Orange	500 tests
22255	CytoTell™ Red	500 tests

3.5 Cell Cycle Assays

The cell cycle has four sequential phases: G0/G1, S, G2, and M. During a cell's passage through cell cycle, its DNA is duplicated in S (synthesis) phase and distributed equally between two daughter cells in M (mitosis) phase. These two phases are separated by two gap phases: G0/G1 and G2. The two gap phases provide time for the cell to grow and double the mass of their proteins and organelles. They are also used by the cells to monitor internal and external conditions before proceeding with the next phase of cell cycle. The cell's passage through cell cycle is controlled by a host of different regulatory proteins.

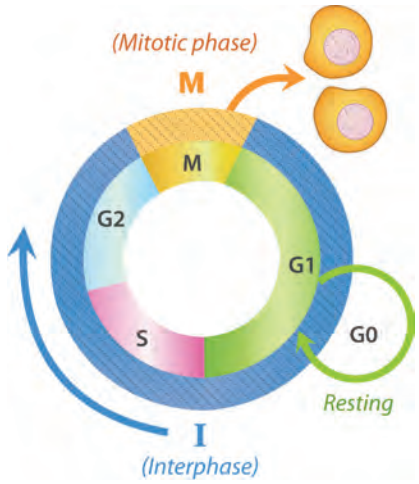

Figure 3.13. Cell division is just one of several stages that a cell goes through during its lifetime. The cell cycle is a repeating series of events that include growth, DNA synthesis, and cell division. The cell cycle in prokaryotes is quite simple: the cell grows, its DNA replicates, and the cell divides. In eukaryotes, the cell cycle is more complicated. The diagram above represents the cell cycle of a eukaryotic cell. As you can see, the eukaryotic cell cycle has several phases. The mitosis phase (M) actually includes both mitosis and cytokinesis. This is when the nucleus and then the cytoplasm divide. The other three phases (G1, S, and G2) are generally grouped together as an interphase. During the interphase, the cell grows, performs routine life processes, and prepares to divide.

AAT Bioquest Cell Meter™ assay kits are a set of tools for monitoring cell viability and proliferation. There are a variety of parameters that can be used for monitoring cell viability and proliferation. In normal cells, DNA density changes depending on whether the cell is growing, dividing, resting or performing its ordinary functions. The progression of the cell cycle is controlled by a complex interplay among various cell cycle regulators. These regulators activate transcription factors, which bind to DNA and turn on or off the production of proteins that result in cell division. Any misstep in this regulatory cascade causes abnormal cell proliferation which underlies many pathological conditions, such as tumor formation. Potential applications for live-cell studies are in the determination of cellular DNA content and cell cycle distribution for detecting variations in growth patterns, for monitoring apoptosis, and for evaluating tumor cell behavior and suppressor gene mechanisms.

Our Cell Meter™ Fluorimetric Cell Cycle Assay Kits are designed to monitor cell cycle progression and proliferation by using our proprietary cell cycle dye in permeabilized and fixed cells. The dye passes through a permeabilized membrane and intercalates into cellular DNA. The signal intensity of the cell cycle dye is directly proportional to DNA content. The percentage of cells in a given sample that are in G0/G1, S and G2/M phases, as well as the cells in the sub-G1 phase prior to apoptosis can be monitored with a flow cytometer.

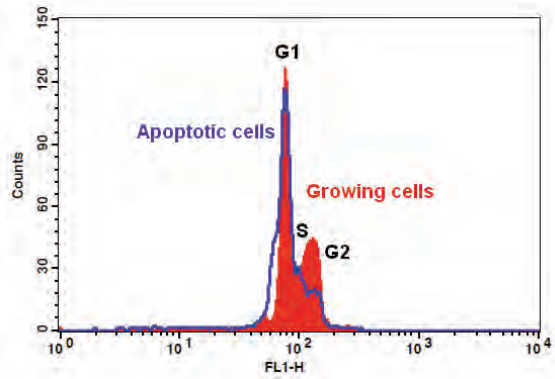

Figure 3.14. DNA profile in growing and camptothecin treated Jurkat cells. Jurkat cells were treated without (red) or with 20 µM camptothecin (blue) in a 37 °C, 5% CO_2 incubator for about 8 hours, and assayed with Cell Meter™ Fluorimetric Cell Cycle Assay kit (Cat# 22841) according to the kit instruction. The fluorescence intensity of Nuclear Green™ LCS1 (Component A) was measured with a FACSCalibur™ flow cytometer using the FL1 channel. In growing Jurkat cells, nuclei stained with Nuclear Green™ LCS1 showed G1, S, and G2 phases (red). In camptothecin treated apoptotic cells (B), the fluorescence intensity of Nuclear Green™ LCS1 was decreased, and both S and G2 phases were diminished.

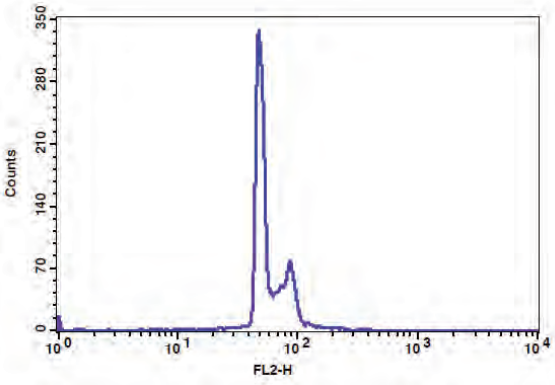

Figure 3.15. DNA profile in growing Jurkat cells. Jurkat cells were dye-loaded with Cell Meter™ Fluorimetric Cell Cycle Assay kit (Cat# 22842) and RNase A for 30 minutes. The fluorescence intensity of Nuclear Red™ CCS1 (Component A) was measured with the FACSCalibur™ (Becton Dickinson, San Jose, CA) flow cytometer using the FL2 channel.

Table 3.5 Cell Cycle Assays

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22841	Cell Meter™ fluorimetric cell cycle assay kit *green fluorescence optimized for flow cytometry*	100 assays	503	526
22842	Cell Meter™ fluorimetric cell cycle assay kit *red fluorescence optimized for flow cytometry*	100 assays	535	617

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Subcellular Compartment Labeling Probes

4

subcellular compartment labeling probes at-a-glance*

	Blue	Green	Red	Deep Red
F-actin	22660, 23100, 23110, 23111	22661, 23115	22664, 23102, 23119, 23122, 23125	23127, 23128, 23129, 23130, 23131
Lysosomes	22655	22651, 22656	22657, 22658	22659
Membrane		22045	22073, 22102, 22190	22070, 22077
Mitochondria		22210, 22666	22211, 22667, 22668, 22673,	22669
Nucleus	17510, 17514, 17520, 17530	17540, 17550	17515, 17542, 17552	17501, 17561

* Products listed by catalog number

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Selective Labeling of Subcellular Compartments

The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context. AAT Bioquest's Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling subcellular organelles such as membranes, lysosomes, mitochondria, nuclei, etc. Cell Navigator™ fluorescence imaging kits provide all the essential components with an optimized cell-labeling protocol. They are suitable for proliferating and non-proliferating cells and can be used for both suspension and adherent cells.

4.1 Cell Nucleus Probes

The nucleus is the largest cellular organelle in animals. In mammalian cells, the average diameter of the nucleus is approximately 6 µm, which occupies about 10% of the total cell volume. Nucleus contains most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins, such as histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome. The function of the nucleus is to maintain the integrity of these genes and to control the activities of the cell by regulating gene expression, therefore, the nucleus is the control center of the cell. The main structures making up the nucleus are the nuclear envelope, a double membrane that encloses the entire organelle and isolates its contents from the cellular cytoplasm, and the nucleoskeleton. Movement of large molecules such as proteins and RNA through the pores is required for both gene expression and the maintenance of chromosomes.

Labeling the Nuclei of Live Cells

Both Hoechst 33258 and Hoechst 33342 are quite soluble in water and relatively nontoxic. They are cell membrane–permeant, minor groove–binding DNA stains that fluoresce bright blue upon binding to DNA. Hoechst 33342 has slightly higher membrane permeability than Hoechst 33258. These Hoechst dyes, which can be excited with the UV spectral lines of the argon-ion laser and by most conventional fluorescence excitation sources, exhibit relatively large Stokes shifts (excitation/emission maxima ~350/460 nm), making them suitable for multicolor labeling experiments. Hoechst 34580 can be better excited by violet laser at 405 nm.

DAPI is quite soluble in water but has limited solubility in PBS buffer. We offer both DAPI chloride and lactate salt. DAPI is an excellent nuclear counterstain, showing a distinct banding pattern in chromosomes. It is one of the most common nuclear dyes for staining nuclei in lives cells in combination with fluorescence imaging or flow cytometry. DAPI demonstrates blue fluorescence upon binding DNA and can be excited with a mercury-arc lamp or with the UV lines of the argon-ion laser. Binding of DAPI to dsDNA produces an ~20-fold fluorescence enhancement, apparently due to the displacement of water molecules from both DAPI and the minor groove.

LDS 751 has its peak excitation at ~543 nm on dsDNA. It can be excited by the argon-ion laser at 488 nm and is particularly useful in multicolor analyses due to its long-wavelength emission maximum (~712 nm). Binding of LDS 751 to dsDNA results in an ~20-fold fluorescence enhancement. LDS 751 is a cell-permeant nucleic acid stain that has been used to discriminate intact nucleated cells from nonnucleated and damaged nucleated cells, as well as to identify distinct cell types in mixed populations of neutrophils, leukocytes and monocytes by flow cytometry.

Nuclear Green™ LCS1, Nuclear Orange™ LCS1, Nuclear Red™ LCS1 and Nuclear Yellow are fluorogenic, DNA-selective and cell-permeant dyes for analyzing DNA content in living cells. The fluorescence of these dyes is significantly enhanced upon binding to DNA. They can be used in fluorescence imaging, microplate and flow cytometry applications. These DNA-binding dyes might be used for multicolor analysis of live cells with proper filter sets.

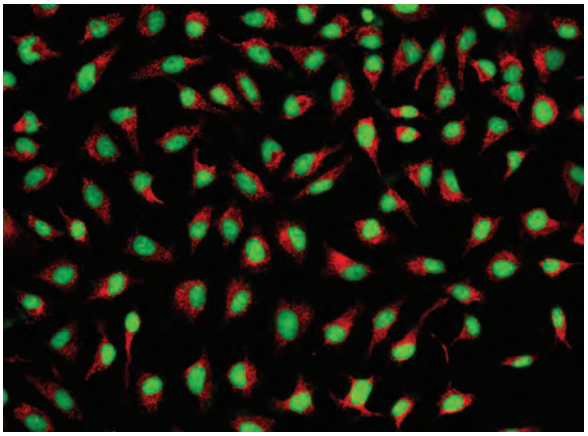

Figure 4.1. Images of live HeLa cells stained with Nuclear Green™ LCS1 (Cat# 17540). The mitochondria of live HeLa cells were stained with red fluorescence Cell Navigator™ Mitochondrion Staining Kit (Cat# 22668).

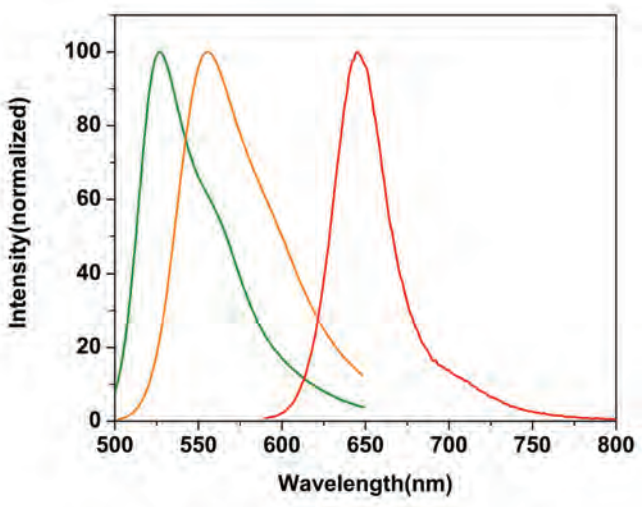

Figure 4.2. The emission spectral comparison of Nuclear Green™ LCS1 (Ex/Em = 503/526 nm, Cat# 17540), Nuclear Orange™ LCS1 (Ex/Em = 514/555 nm, Cat#17541), and Nuclear Red™ LCS1 (Ex/Em = 622/645 nm, Cat# 17542) bound to calf thymus DNA.

Labeling the Nuclei of Dead Cells

Propidium iodide (PI) belongs to the same chemical class of ethidium bromide. As in the case of ethidium bromide, the fluorescence of PI is enhanced by 20-30-fold upon binding to nucleic acids. The fluorescence excitation maximum is red-shifted by 30–40 nm and the fluorescence emission maximum blue-shifted by 15 nm or so. PI also binds to RNA as DAPI and acridine orange. PI is cell-impermeable and commonly used for identifying dead cells in a population of cells and as a counterstain in multicolor fluorescent techniques. It can also be used to differentiate necrotic, apoptotic and normal cells. It is suitable for fluorescence microscopy, flow cytometry and fluorometry.

7-Amino actinomycin D (7-AAD) is another non-permeant dye that can be used to identify non-viable cells. 7-AAD is typically used with a flow cytometer. Cells with damaged plasma membranes or with impaired/no cell metabolism are unable to prevent the dye from entering the cell. Once inside the cell, the dyes bind to intracellular DNA producing highly fluorescent adducts which identify the cells as non-viable. 7-AAD is excited by the 488 nm laser line of an argon laser with fluorescence detected above 650 nm. Although the emission intensity of 7-AAD is lower than that of PI, the longer wavelength emission may make it more useful for multiplexing assays in combination with other 488 nm-excited fluorochromes such as FITC and PE.

Nuclear Green™ DCS1, Nuclear Orange™ DCS1 and Nuclear Red™ DCS1 are fluorogenic, DNA-selective and cell-impermeant dyes for analyzing DNA content in dead, fixed or apoptotic cells. As the LCS1 reagents, the fluorescence of the DCS1 dyes is significantly enhanced upon binding to DNA. They can be used in fluorescence imaging, microplate and flow cytometry applications. These DNA-binding dyes might be used for multicolor analysis of dead, fixed or apoptotic cells with proper filter sets.

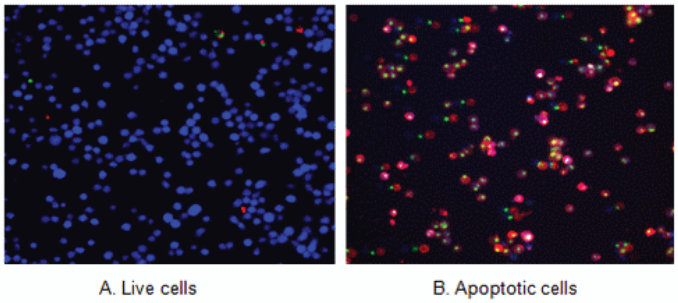

Figure 4.3. The detection of binding activity of Apopxin™ Deep Red to phosphatidylserine in Jurkat cells. The fluorescence image showing cells that are live (blue, stained by CytoCalcein™ Violet 450, Cat# 22012), apoptotic (red, stained by Apopxin™ Deep Red), and necrotic (green, stained by Nuclear Green™ DCS1, Cat# 17550) induced by 1µM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope using the violet, Cy5® and FITC channel respectively. A: Non-induced control cells; B: Triple staining of staurosporine-induced cells.

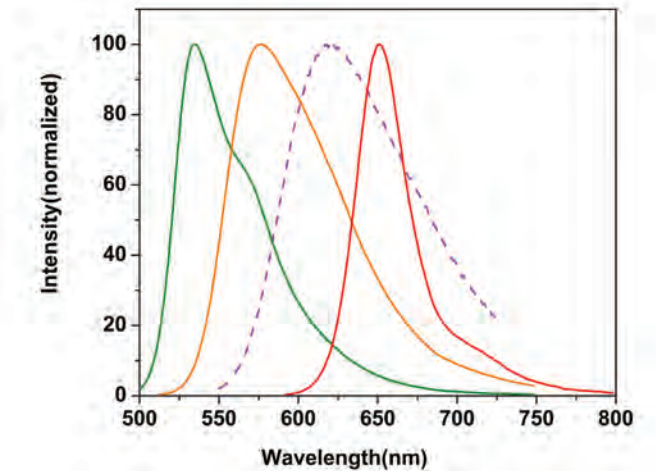

Figure 4.4. The emission spectral comparison of Nuclear Green™ DCS1 (Ex/Em = 503/526 nm, Cat# 17550), Nuclear Orange™ DCS1 (Ex/Em = 514/555 nm, Cat# 17551), and Nuclear Red™ DCS1 (Ex/Em = 622/645 nm, Cat# 17552) in the presence of calf thymus DNA. The dotted line is emission spectrum of propidium iodide bound to DNA (Ex/Em = 535/617 nm, Cat# 17515).

Table 4.1 Cell Nuclear Stains

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
17501	7-AAD [7-Aminoactinomycin D]	1 mg	546	647
17510	DAPI [4,6-Diamidino-2-phenylindole, dihydrochloride] *UltraPure grade*	10 mg	358	461
17520	Hoechst 33258 *UltraPure grade*	100 mg	352	461
17530	Hoechst 33342 *UltraPure grade*	100 mg	350	461
17537	Hoechst 34580 *UltraPure grade*	5 mg	368	437
17561	LDS 751	25 mg	543	712
17550	Nuclear Green™ DCS1	5 mM	503	526
17540	Nuclear Green™ LCS1	5 mM	503	526
17551	Nuclear Orange™ DCS1	5 mM	528	576
17541	Nuclear Orange™ LCS1	5 mM	514	555
17552	Nuclear Red™ DCS1	5 mM	631	651
17542	Nuclear Red™ LCS1	5 mM	622	645
17515	Propidium iodide *UltraPure grade*	25 mg	535	617

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4.2 Cell Membrane Probes

The cell membrane is a biological membrane that separates the interior of all cells from the outside environment. The cell membrane is selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells. The basic function of the cell membrane is to protect the cell from its surroundings. It consists of the lipid bilayer with embedded proteins. Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling and serve as the attachment surface for several extracellular structures, including the cell wall, glycocalyx and intracellular cytoskeleton.

The cationic carbocyanine dyes is one of the major classses of cell membrane probes. They accumulate on hyperpolarized membranes and are translocated into the lipid bilayer. Aggregation within the confined membrane interior usually results in decreased fluorescence, although the magnitude and even the direction of the fluorescence response are strongly dependent on the concentration of the dye and its structural characteristics. $DiOC_6(3)$ and $DiOC_5(3)$ have been the most widely used carbocyanine dye for membrane potential measurements. In flow cytometry measurements, the detected intensity of carbocyanine fluorescence is dependent not only on the membrane potential but also on cell size. In some cases, measurements of forward light scatter have been used to normalize the optical changes for cell size variability.

Table 4.2 Cell Membrane Probes

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22033	DiD	10 mL	644	663
22101	DiI iodide	100 mg	549	565
22102	DiI perchlorate	100 mg	549	565
22103	DiI triflate	100 mg	549	565
22056	$DiIC_1(5)$ iodide	25 mg	638	658
22050	$DiIC_{12}(3)$-DS	5 mg	555	570
22035	$DiIC_{12}(3)$ perchlorate	25 mg	549	565
22051	$DiIC_{12}(5)$-DS	5 mg	650	670
22044	$DiIC_{16}(3)$ perchlorate	25 mg	549	565
22052	$DiIC_{18}(3)$-DS	5 mg	555	570
22054	$DiIC_{18}(5)$-DS	5 mg	650	670
22066	DiO perchlorate	25 mg	484	501
22038	$DiOC_2(3)$ iodide	25 mg	482	497
22039	$DiOC_3(3)$ iodide	25 mg	482	497
22045	$DiOC_5(3)$ iodide	25 mg	482	504
22046	$DiOC_6(3)$ iodide	25 mg	482	504
22040	$DiOC_7(3)$ iodide	25 mg	482	504
22042	$DiOC_{16}(3)$ perchlorate	25 mg	484	501
22070	DiR iodide	25 mg	748	780
22073	$DiSC_2(3)$	25 mg	560	571
22077	$DiSC_2(7)$	25 mg	770	790
22076	$DiSC_3(5)$	25 mg	660	675
22190	Nile Red *UltraPure grade*	25 mg	552	636

4.3 Lysosome Staining Probes

Lysosomes are cellular organelles that contain acid hydrolase enzymes that break down waste materials and cellular debris. Lysosomes digest excess or worn-out organelles, food particles, and engulf viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at the pH they require. Lysosomes fuse with autophagic vacuoles and dispense their enzymes into the autophagic vacuoles, digesting their contents. The size of a lysosome varies from 0.1–1.2 µm. At pH 4.8, the interior of the lysosomes is acidic compared to the slightly basic cytosol (pH 7.2). The lysosome maintains this pH differential by pumping protons from the cytosol across the membrane via proton pumps and chloride ion channels. The lysosomal membrane protects the cytosol, and therefore the rest of the cell, from the degradative enzymes within the lysosome. The cell is additionally protected from any lysosomal acid hydrolases that drain into the cytosol, as these enzymes are pH-sensitive and do not function well or at all in the alkaline environment of the cytosol. This ensures that cytosolic molecules and organelles are not lysed in case there is leakage of the hydrolytic enzymes from the lysosome.

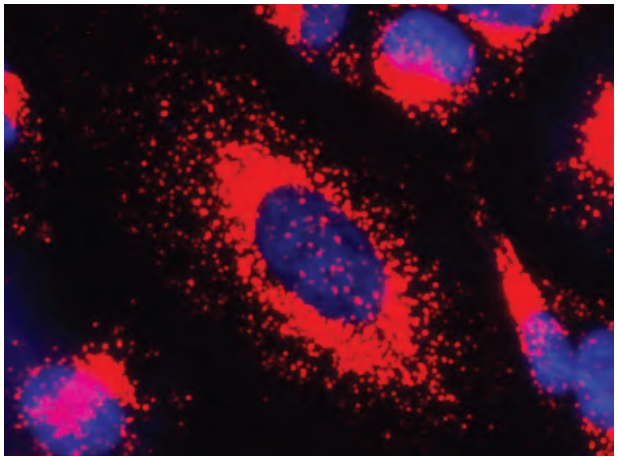

Figure 4.5. Image of HeLa cells stained with Cell Navigator™ Lysosome Staining Kit (Cat# 22658) in a Costar 96-well black wall/clear bottom plate. TRITC filter was used for imaging. Nuclei were stained with Hoechst 33342 (Cat# 17530) .

Our Cell Navigator™ Lysosome Staining Kits are designed to label lysosomes of live cells with LysoBrite™ dyes, our proprietary lysotropic indicators which selectively accumulate in lysosomes probably via the lysosome pH gradient. The LysoBrite™ dyes are hydrophobic compounds that easily permeate intact live cells, and trapped in lysosomes after they get into cells. Their fluorescence is significantly enhanced upon entering lysosomes. This key feature significantly reduces the staining background and makes the assay kits useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The Cell Navigator™ staining kits are suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. The labeling protocols are robust, requiring minimal hands-on time. The kits can be readily adapted for many types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope.

Key Features of Cell Navigator™ Lysosome Staining Kits

- *Minimal cytotoxicity (no cell toxicity observed)*
- *Multicolor wavelengths for multiplexing*
- *Enhanced signal intensity*
- *Extraordinarily high photostability*
- *Excellent cellular retention (more than 6 passages for cell tracking in Hela cells)*
- *Fixable (cell staining pattern survives fixation)*

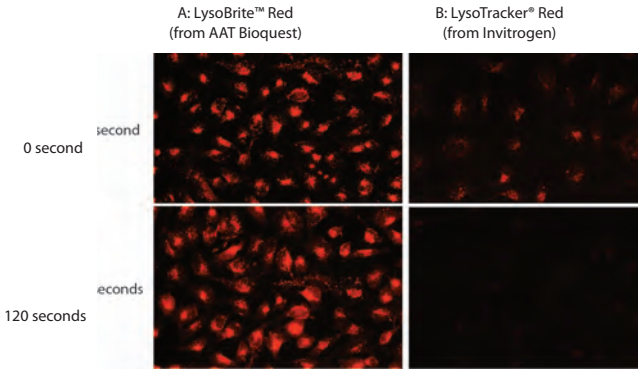

Figure 4.6. Images of HeLa cells stained with A: Cell Navigator™ Lysosome Staining Kit (Cat# 22658), B: LysoTracker® Red DND-99 (from Invitrogen) in a Costar black wall/clear bottom 96-well plate. The signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

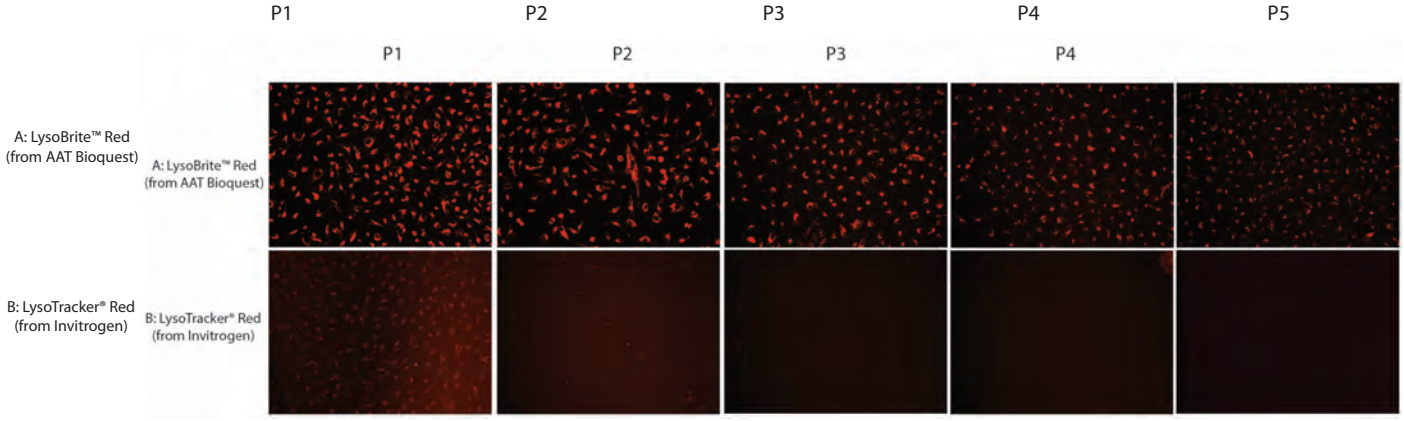

Figure 4.7. Images of HeLa cells stained with A: Cell Navigator™ Lysosome Staining Kit (Top, from AAT Bioquest, Cat# 22658), B: LysoTracker® Red DND-99 (Bottom, from Invitrogen) in a Costar black wall/clear bottom 96-well plate. The signals were compared at 5 cell passages (P1, P2, P3, P4 and P5) respectively using an Olympus fluorescence microscope.

Table 4.3 Lysosome Staining Probes

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22655	Cell Navigator™ lysosome staining kit *blue fluorescence*	500 assays	353	442
22659	Cell Navigator™ lysosome staining kit *deep red fluorescence*	500 assays	596	619
22656	Cell Navigator™ lysosome staining kit *green fluorescence*	500 assays	450	505
22651	Cell Navigator™ lysosome staining kit *green fluorescence with 405 nm excitation*	500 assays	405	505
22652	Cell Navigator™ lysosome staining kit *NIR fluorescence*	500 assays	636	650
22657	Cell Navigator™ lysosome staining kit *orange fluorescence*	500 assays	542	556
22658	Cell Navigator™ lysosome staining kit *red fluorescence*	500 assays	575	597
22642	LysoBrite™ Blue	500 tests	353	442
22646	LysoBrite™ Deep Red	500 tests	596	619
22643	LysoBrite™ Green	500 tests	450	505
22641	LysoBrite™ NIR	500 tests	636	650
22644	LysoBrite™ Orange	500 tests	542	556
22645	LysoBrite™ Red	500 tests	575	597

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4.4 Mitochondrial Staining Probes

The mitochondrion is a membrane-enclosed organelle found in most eukaryotic cells. These organelles range from 0.5 to 1.0 µm in diameter. Mitochondria generate most of the cell's supply of ATP as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in other tasks such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process. Several characteristics make mitochondria unique. The number of mitochondria in a cell varies widely by organism and tissue type. Many cells have only a single mitochondrion, whereas others can contain several thousand mitochondria. The organelle is composed of compartments that carry out specialized functions. Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome.

Cell Navigator™ Mitochondrion Staining Kits are designed to label mitochondria of live cells with a full set of fluorescence colors. The kits use proprietary dyes that selectively accumulate in mitochondria probably via the mitochondrial membrane potential gradient. The mitochondrial indicators are retained in mitochondria for a long time and show good photostability. This key feature significantly increases the staining efficiency.

Besides our robust Cell Navigator™ Mitochondrion Staining Kits, we also offer some common mitochondrial stains and probes (such as JC-1, TMRE, TMRM and rhodamine 123 etc). Both cyanine and rhodamine mitochondrial stains are positively charged, thus they are selectively located in mitochondria through the mitochondrial membrane potential. Among them JC-1 is primarily used for monitoring the mitochondrial membrane potentials of apoptotic cells. We offer JC-10™ as a superior replacement to JC-1. JC-10 has better water solubility and larger response than JC-1. TMRE and TMRM are primarily used for monitoring cell mitochondrial membrane potential changes.

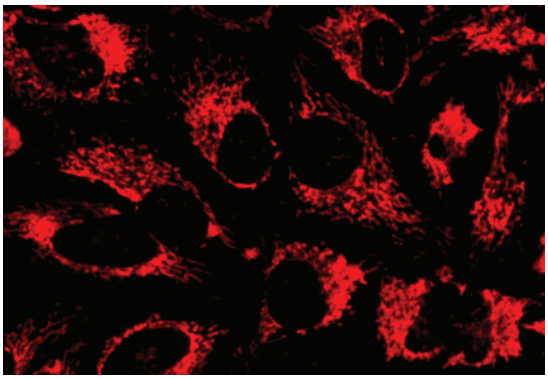


Figure 4.8. Image of HeLa cells stained with Cell Navigator™ Mitochondrion Staining Kit (Cat# 22668) in a Costar black 96-well plate.

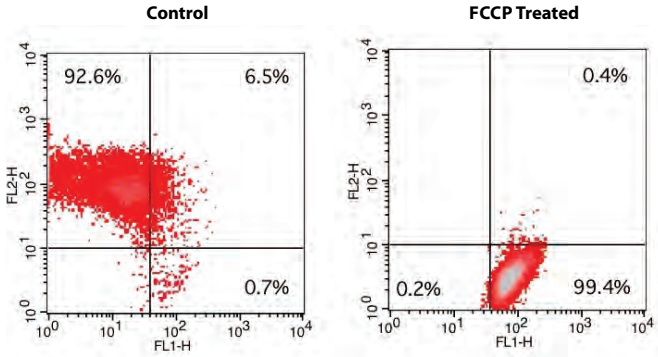


Figure 4.9. FCCP-induced mitochondrial membrane potential changes in Jurkat cells. Jurkat cells were dye loaded with JC-10™ dye-loading solution along with DMSO (left) or 20 µM FCCP (right) for 10 minutes. The fluorescence intensities for both J-aggregates and monomeric forms of JC-10™ were measured with FACSCalibur™ flow cytometer (Becton Dickinson) using FL1 and FL2 channels after compensation.

Table 4.4 Mitochondrial Staining Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
22666	Cell Navigator™ mitochondrion staining kit *green fluorescence*	500 assays	498	520
22669	Cell Navigator™ mitochondrion staining kit *NIR fluorescence*	500 assays	640	659
22667	Cell Navigator™ mitochondrion staining kit *orange fluorescence*	500 assays	545	575
22673	Cell Navigator™ mitochondrion staining kit *orange fluorescence with 405 nm excitation*	500 assays	399	550
22668	Cell Navigator™ mitochondrion staining kit *red fluorescence*	500 assays	575	600
22200	JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide]	5 mg	515	529
22201	JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide]	50 mg	515	529
22204	JC-10 *superior alternative to JC-1*	5x100 µL	510	525
22210	Rhodamine 123	25 mg	507	529
22220	TMRE [tetramethylrhodamine ethyl ester]	25 mg	549	574
22221	TMRM [tetramethylrhodamine methyl ester]	25 mg	549	573

4.5 iFluor™ Phalloidin Conjugates for Labeling F-actins

Actin is a globular, roughly 42-kDa protein found in almost all eukaryotic cells. It is also one of the most highly-conserved proteins, differing by no more than 20% in species as diverse as algae and humans. Actin is the monomeric subunit of two types of filaments in cells: microfilaments, one of the three major components of the cytoskeleton, and thin filaments, part of the contractile apparatus in muscle cells. Thus, actin participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, as well as the establishment and maintenance of cell junctions and cell shape.

AAT Bioquest offers a variety of fluorescent phalloidin derivatives with different colors for multicolor imaging of F-actin. Fluorescent derivatives of phalloidin have turned out to be enormously useful in localizing actin filaments in living or fixed cells as well as for visualizing individual actin filaments *in vitro*. Fluorescent phalloidin derivatives have been used as an important tool in the study of actin networks at high resolution. Used at nanomolar concentrations, phalloidin derivatives are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. Phalloidin binds to actin filaments much more tightly than to actin monomers, leading to a decrease in the rate constant for the dissociation of actin subunits from filament ends, essentially stabilizing actin filaments through the prevention of filament depolymerization. Moreover, phalloidin is found to inhibit the ATP hydrolysis activity of F-actin. Phalloidin functions differently at various concentrations in cells. When introduced into the cytoplasm at low concentrations, phalloidin recruits the less polymerized forms of cytoplasmic actin as well as filamin into stable "islands" of aggregated actin polymers, yet it does not interfere with stress fibers, i.e. thick bundles of microfilaments.

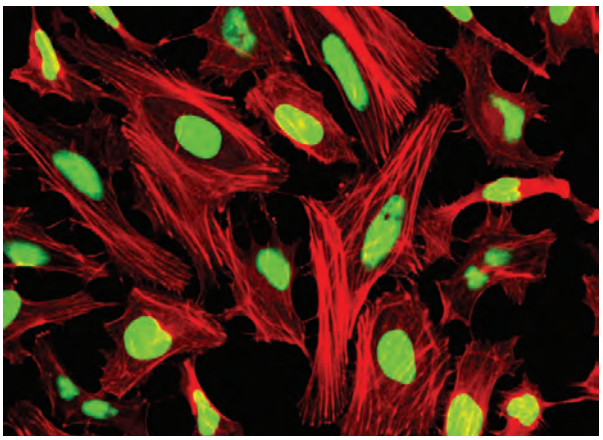


Figure 4.10. Image of HeLa cells. Actin filaments were stained with Phalloidin-iFluor™ 680 conjugate (red, Cat# 23128), and nuclei were stained with Nuclear Green™ DCS1 (green, Cat#17550).

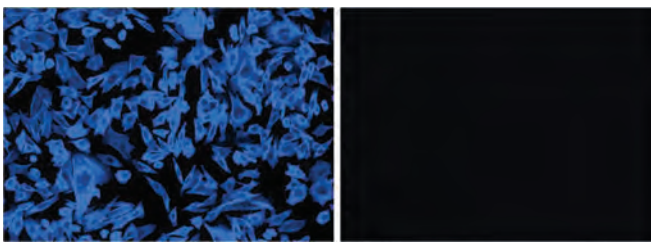
Table 4.5 Phalloidin-iFluor™ Conjugates

Cat #	Product Name	Size	Ex (nm)	Em (nm)
23100	Phalloidin-AMCA conjugate	300 tests	353	442
23103	Phalloidin-California Red conjugate	300 tests	583	605
23101	Phalloidin-Fluorescein conjugate	300 tests	492	518
23110	Phalloidin-iFluor™ 350 conjugate	300 tests	353	442
23111	Phalloidin-iFluor™ 405 conjugate	300 tests	400	421
23115	Phalloidin-iFluor™ 488 conjugate	300 tests	493	517
23116	Phalloidin-iFluor™ 514 conjugate	300 tests	520	547
23117	Phalloidin-iFluor™ 532 conjugate	300 tests	542	558
23119	Phalloidin-iFluor™ 555 conjugate	300 tests	556	574
23122	Phalloidin-iFluor™ 594 conjugate	300 tests	590	618
23125	Phalloidin-iFluor™ 633 conjugate	300 tests	634	649
23127	Phalloidin-iFluor™ 647 conjugate	300 tests	650	665
23128	Phalloidin-iFluor™ 680 conjugate	300 tests	681	698
23129	Phalloidin-iFluor™ 700 conjugate	300 tests	692	708
23130	Phalloidin-iFluor™ 750 conjugate	300 tests	752	778
23131	Phalloidin-iFluor™ 790 conjugate	300 tests	787	808
23102	Phalloidin-Tetramethylrhodamine conjugate	300 tests	546	575

Cell Navigator™ F-Actin Labeling Kits are designed to label F-actins in fixed cells. The kits use fluorescent phalloidin conjugates that are selectively bound to F-actins. The fluorescent phalloidin conjugates are high-affinity probes for F-actins. When used at nanomolar concentrations, phallotoxins are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. The kits provide all the essential components with an optimized staining protocol, which is robust requiring minimal hands-on time.

Table 4.6 F-actin Labeling Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22660	Cell Navigator™ F-actin labeling kit *blue fluorescence*	1 kit	353	442
22661	Cell Navigator™ F-actin labeling kit *green fluorescence*	1 kit	498	520
22663	Cell Navigator™ F-actin labeling kit *orange fluorescence*	1 kit	546	575
22664	Cell Navigator™ F-actin labeling kit *red fluorescence*	1 kit	583	603



A B

Figure 4.11. Images of fixed CPA cells stained with Cell Navigator™ F-Actin Labeling Kit (Cat# 22660) in a 96-well Costar black wall/clear bottom plate A: Label the cells with 1X Phalloidin-iFluor™ 350 for 30 minutes only. B: Treat the cells with phalloidin for 10 minutes, then stain them with 1X Phalloidin-iFluor™ 350 for 30 minutes.

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Reporter Gene Analysis 5

reporter gene assay kits at-a-glance*

Reporter Gene	Fluorescence	Luminescence
Beta-Galactosidase Assay (LacZ)	12601	
Firefly Luciferase Reporter Gene		12518, 12519 &12520
Gaussia Luciferase Reporter Gene		12530, 12531 &12532
Renilla Luciferase Reporter Gene		12535, 12536 &12537

* products listed by catalog number

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Reporter Gene Analysis

5.1 Firefly Luciferase Reporter Gene Assay

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

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

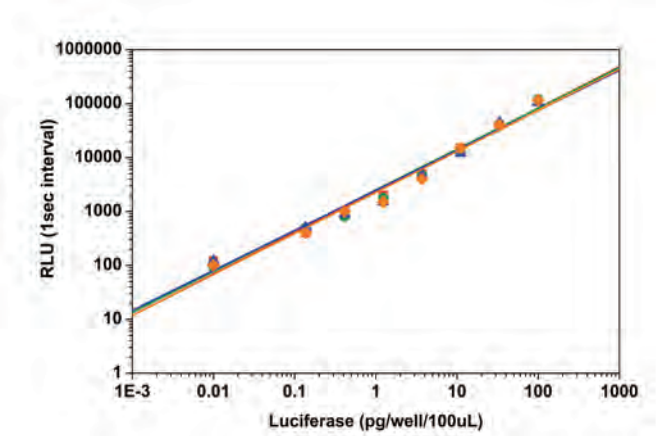

Figure 5.1. Luciferase dose responses were measured with Amplite™ Luciferase Reporter Gene Assay Kit (Cat# 12518). The kit can detect as low as 0.1 pg/well luciferase with 20-minute to 5-hour incubation without losing signal intensity. The integration time is 1 second. The half life is more than 4 hours.

5.2 Gaussia Luciferase Reporter Gene Assay

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

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

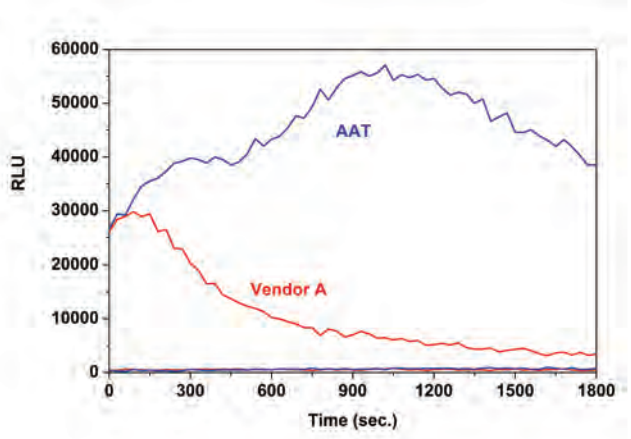

Figure 5.2. Secreted Gaussia luciferase culture medium was measured with Amplite™ Gaussia Luciferase Reporter Gene Assay Kit (blue line, Cat# 12530) and a commercially available Gaussia Luciferase Assay Kit (red line) respectively in a 96-well white plate using a NOVOstar plate reader (BMG Labtech).

Table 5.1 Firefly and Gaussia Luciferase Reporter Gene Assay Kits

Cat. #	Product Name	Size	Em (nm)
12530	Amplite™ Gaussia luciferase reporter gene assay kit	1 plate	466
12531	Amplite™ Gaussia luciferase reporter gene assay kit	10 plates	466
12532	Amplite™ Gaussia luciferase reporter gene assay kit	100 plates	466
12518	Amplite™ luciferase reporter gene assay kit *bright glow*	1 plate	533
12519	Amplite™ luciferase reporter gene assay kit *bright glow*	10 plates	533
12520	Amplite™ luciferase reporter gene assay kit *bright glow*	100 plates	533

5.3 Renilla Luciferase Reporter Gene Assay

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

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

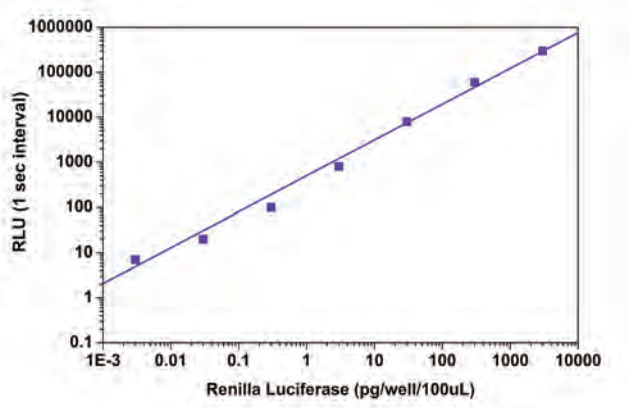

Figure 5.3. Renilla Luciferase dose responses were measured with Amplite™ Renilla Luciferase Reporter Gene Assay Kit (Cat# 12535) in a 96-well solid black plate with a NOVOstar plate reader (BMG Labtech). As low as 1pg/mL (0.1pg/well/100uL) Renilla luciferase was detected with 30-minutes incubation (n=3).

Table 5.2 Renilla Luciferase Reporter Gene Assay Kits

Cat. #	Product Name	Size	Em (nm)
12535	Amplite™ Renilla luciferase reporter gene assay kit *bright glow*	1 plate	466
12536	Amplite™ Renilla luciferase reporter gene assay kit *bright glow*	10 plates	466
12537	Amplite™ Renilla luciferase reporter gene assay kit *bright glow*	100 plates	466

5.4 Fluorimetric Beta-Galactosidase Assay

E. coli β-galactosidase is a 464 kD tetramer. Each unit of β-galactosidase consists of five domains, the third of which is the active site. It is an essential enzyme in cells. Deficiencies of this enzyme can result in galactosialidosis or Morquio B syndrome. In E. coli, β-galactosidase is produced by the activation of LacZ operon. Detection of LacZ expression has become routine to the point of detection of as few as 5 copies of β-galactosidase per cell.

Amplite™ Fluorimetric Beta-Galactosidase Assay Kit uses the fluorogenic fluorescein digalactoside (FDG) galactosidase substrate that can sensitively distinguish LacZ+ from LacZ- cells. The non-fluorescent substate generates the strongly fluorescent fluorescein upon reaction with galactosidase. It can be used either for detecting galactosidase conjugates in ELISA type assay systems or for monitoring LacZ gene expression in cells. FDG used in the kit is not fluorescent. The galactosidase induced cleavage of FDG gives fluorescein that has the spectra of Ex/Em = 490/515 nm, which can be detected with most fluorescence instruments equipped with a FITC filter set. The kit comes with all the essential components with an optimized assay protocol. It can be used with a fluorescence microplate reader, a fluorescence microscope, or a flow cytometer. It might also be used for screening galactosidase inhibitors or inducers.

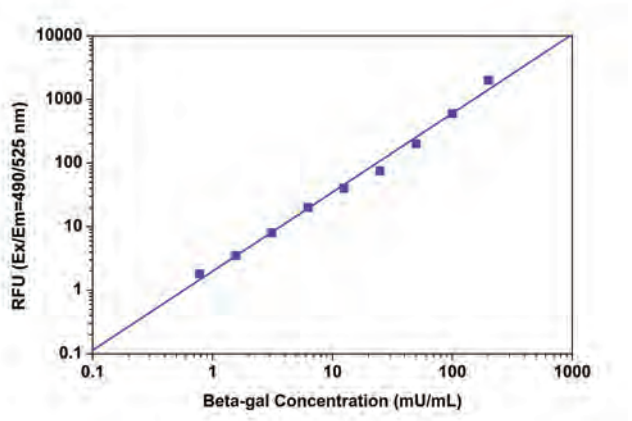

Figure 5.4. ß-galactosidase dose responses were measured with Amplite™ Fluorimetric Beta-Galactosidase Assay Kit (Cat# 12601) in a Costar 96-well black solid plate using Gemini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU/well ß-galactosidase was detected with 30-minute incubation.

Table 5.3 Fluorimetric Beta-Galactosidase Assay

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
12601	Amplite™ fluorimetric Beta-galactosidase assay kit *green fluorescence*	500 assays	490	514

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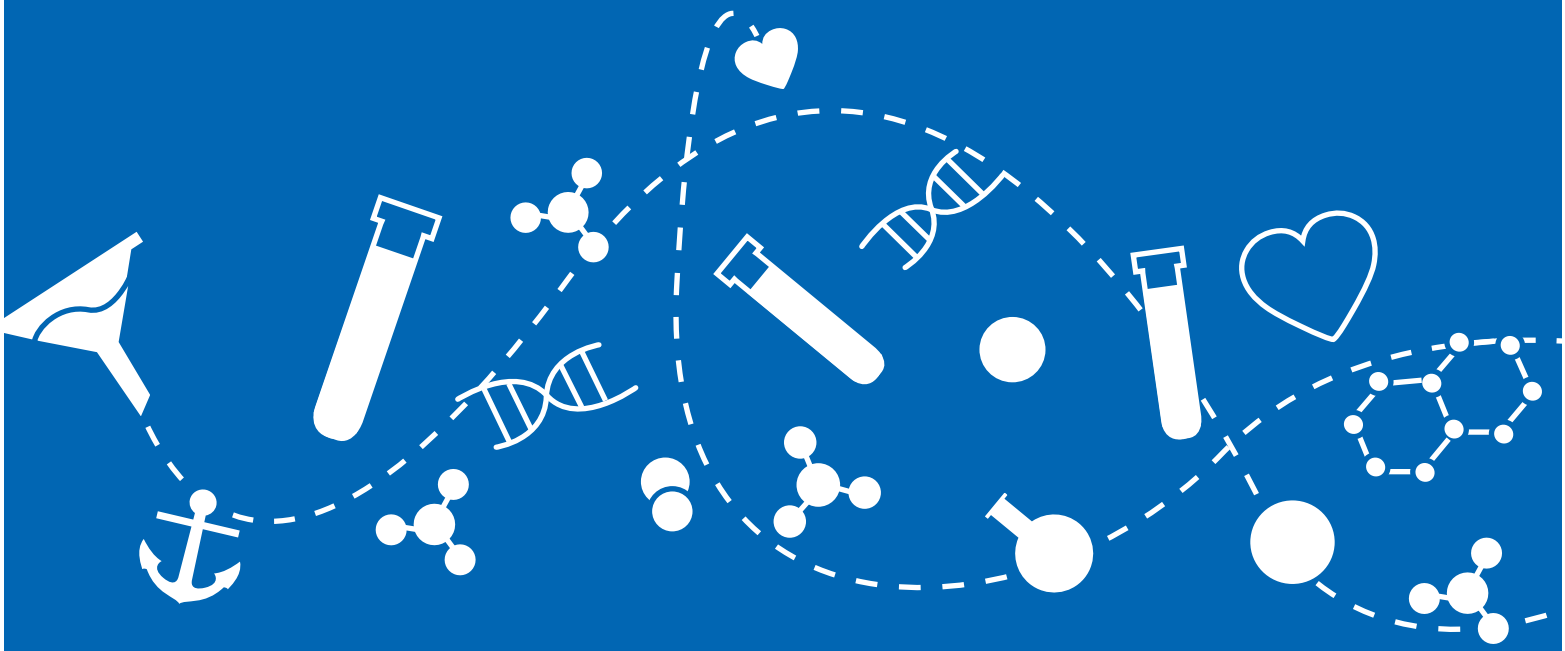
SIE HABEN DIE VISION,
WIR HABEN DIE SUBSTANZ.



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