Fluorescent Labeling Probes & Kits

iFLUOR™
OPTIMIZED FOR ANTIBODIES

mFLUOR™
OPTIMIZED FOR FLOW CYTOMETRY

trFLUOR™
OPTIMIZED FOR TR-FRET

biomol
SIE HABEN DIE VISION,
WIR HABEN DIE SUBSTANZ.

AAT Bioquest®
Advancing Assay & Test Technologies

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Your Distributor:
BIOMOL GmbH
Waidmannstraße 35
22769 Hamburg · Germany
info@biomol.de · www.biomol.de · www.biomol.com
Fon: +49 (0)40-853 260 0
Fax: +49 (0)40-853 260 22

TOLL FREE IN GERMANY:
Fon: 0800-246 66 51
Fax: 0800-246 66 52

Trademarks of AAT Bioquest

AAT Bioquest®
California Red™
iFluor™
mFluor™
Nuclear Blue™
Nuclear Green™
Nuclear Orange™
Nuclear Red™
ReadiLink™
ReadiView™
Tide Fluor™
Tide Quencher™
trFluor™

Trademarks of Other Companies

Alexa Fluor® (Invitrogen)
Cy3®, Cy5®, Cy5.5® and Cy7® (GE Healthcare)
DyLight™ (ThermoFisher)
IRDye® (LI-COR)
Krome Orange™ (Beckman Coulter)
Lightning-Link® (Innova Biosciences)
Pacific Blue® and Pacific Orange® (Invitrogen)
Texas Red®(Invitrogen)

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™, 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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Fluorescence Labeling Principles 2

Fluorescence Labeling Methods at-a-glance

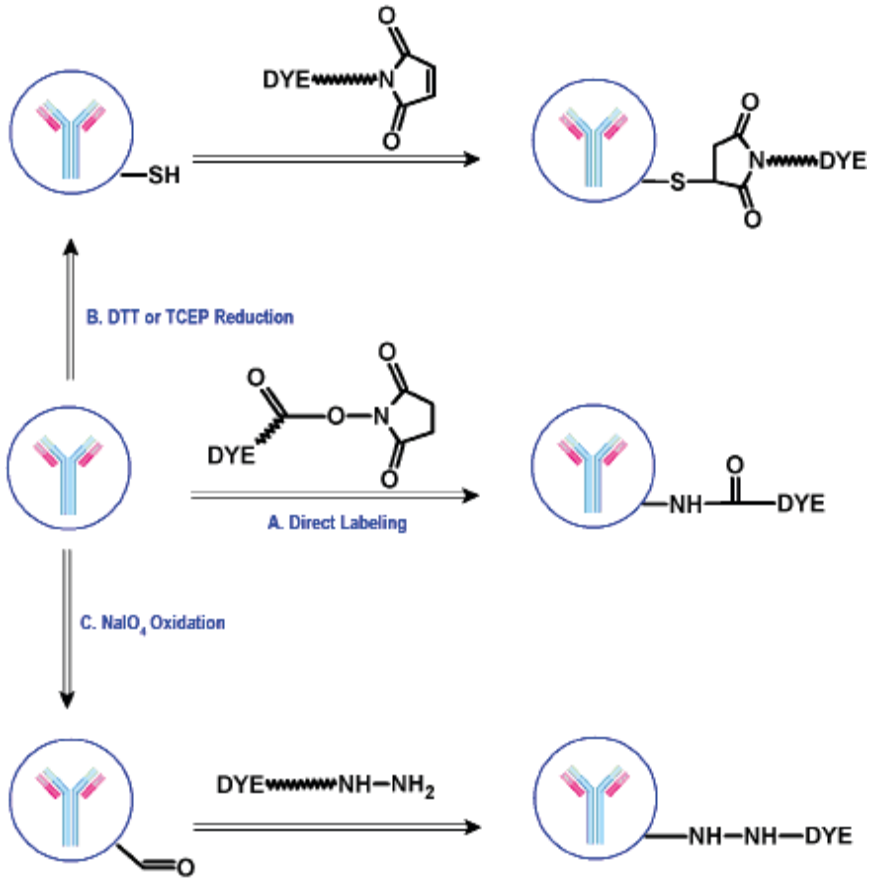

Figure 2.1 Three common methods used for labeling antibodies with fluorescent dyes. (A). Direct labeling through amino groups. This method is convenient and gives the highest dye/protein ratio. However, the overlabeling may reduce the specificity and affinity of the antibody to be labeled. (B). Reduction method. Antibodies are first treated with DTT (or TCEP) to generate a SH group that reacts with a dye maleimide. The resulted conjugate has generally good specificity and affinity. (C). Oxidation method. The antibodies are first treated with $NaIO_4$ to generate an aldehyde group that reacts with a dye hydrazide. This method generally gives lower dye/protein ratio, thus the resulted dye-protein conjugate tends to be less brighter than the two other methods.

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Optimized Fluorescence Labeling Solutions

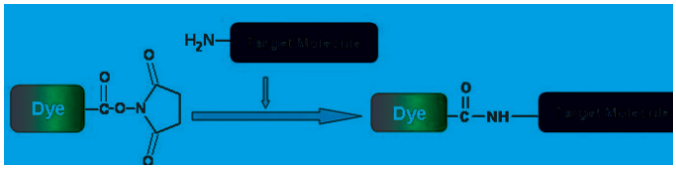
Fluorescence is the result of a three-stage process that occurs in certain molecules (generally polyaromatic hydrocarbons or heterocycles) called fluorophores or fluorescent dyes. A fluorescent probe is a fluorophore designed to localize within a specific region of a biological specimen or to respond to a specific stimulus. Fluorescent probes enable researchers to detect particular components of complex biomolecular assemblies (such as live cells) with exquisite sensitivity and selectivity. Reactive fluorescent dyes are widely used to modify peptides, proteins (in particular, antibodies), oligonucleotides, nucleic acids, carbohydrates and other biological molecules. In general, the preferred bioconjugates should have high fluorescence quantum yields and retain the biological activities of the unlabeled biomolecules.

A number of fluorescent dyes have been developed and commercialized for labeling biomolecules. Among them, FITC might be the most popular one although it has certain limitations, e.g., pH dependence, low photostability and short wavelength, etc. Alexa Fluor® dyes have been used for labeling proteins and other biomolecules with improved properties over the classic dyes such as fluorescein and rhodamine molecules. However, the extra-ordinary high costs of Alexa Fluor® dyes keep them from the certain applications that require a large amount of dye such as labeling peptides and oligos. In addition, Alexa Fluor® dyes do not provide a significant benefit for labeling peptides, oligo and other small molecules.

AAT Bioquest offers iFluor™, mFluor™ and trFluor™ dyes, a complete set of fluorescent labeling dyes, tiered and optimized for a variety of particular applications with significantly reduced cost. Our iFluor™ dyes are optimized for labeling antibodies and other biopolymers including nucleic acids and carbohydrates. The iFluor™ dyes demonstrate strong fluorescence, high photostability and pH independence on proteins and other biopolymers. Our mFluor™ fluorescent labeling dyes are developed specifically for flow cytometry-based applications. Our trFluor™ dyes are ideal for TR-FRET-based applications and other time-resolved fluorescence-based assays with superior performance and reduced cost.

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

Dye Carboxy Acids and Their Succinimidyl Esters



Among the reactive dyes, amine-reactive dyes are most often used to prepare various bioconjugates for immunochemistry, histo-chemistry, fluorescence in situ hybridization (FISH), cell tracing, receptor binding and other biological applications since amino groups are either abundant or easily introduced into biomolecules. Thiol-reactive reagents are frequently used to develop probes for investigating some particular protein structures and functions. Some amine-containing fluorescent reagents are also used to modify biomolecules, in particular, to label glycoproteins. Besides amine-reactive and thiol-reactive dyes, AAT Bioquest also offers dye azides and alkynes for the rapidly growing applications of click chemistry.

Succinimidyl esters (SE) are proven to be the best reagents for amine modifications because the amide bonds formed are essentially identical to, and as stable as the natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines. A few factors should be considered when SE compounds are used for conjugation reactions:

- Reaction Solvents: For the most part, reactive dyes are hydro-phobic molecules and should be dissolved in anhydrous dimethyl-formamide (DMF) or dimethylsulfoxide (DMSO).

- Reaction pH: The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react

Table 2.1 Optimized Fluorescence Labeling Solutions Offered by AAT Bioquest

Targets to be labeled	Dye Selection	Benefits
Antibodies, Proteins and Nucleic Acids	iFluor™ dyes	• Superior performance to the classic dyes • Uncompromised performance with significant cost saving (Compared to Alexa Fluor® and DyLight™ dyes)
Oligos and Peptides	Tide Fluor™ dyes	• Better performance than the classic dyes • Uncompromised performance with a fraction of cost (Compared to Alexa Fluor® and DyLight™ dyes)
Flow Cytometry Applications	mFluor™ dyes	• Enable multicolor detection optimized for flow cytometry
TR-FRET Applications	trFluor™ dyes	• No enhancers required • Multiple reactive forms available

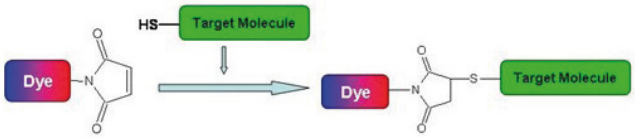
with non-protonated aliphatic amine groups, including the terminal amines of proteins and the ε-amino groups of lysines. Thus amine acylation reactions are usually carried out above pH 7.5. Protein modifications by succinimidyl esters can typically be done at pH 8.5-9.5.

- Reaction Buffers: Buffers that contain free amines such as Tris, glycine and thiol compounds must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed (such as dialysis) before performing dye conjugations.

- Reaction Temperature: Most of the conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

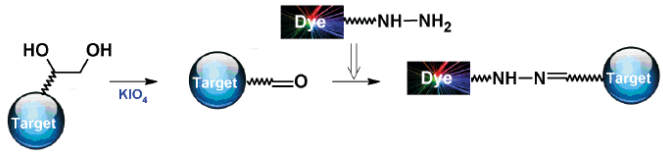
For labeling biopolymers it is quite critical to properly control the degree of substitution (DOS). A high degree of labeling may significantly decrease the water solubility and binding affinity/specificity of the target biomolecules. Although conjugating dyes to biomolecules is usually easy, preparing the optimal conjugate may require extensive experimentation. Fortunately there are some excellent publications that may provide you some important guidelines.

Dye Maleimides



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

Carbonyl-Reactive (Amine-Containing) Fluorescent Dyes and Their Applications



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

Amine-containing dyes are also used to modify biopolymers (such as proteins) using water-soluble carbodiimides (such as EDC) to convert the carboxy groups of the biopolymers into amide groups. Either NHS or NHSS may be used to improve the coupling efficiency of EDC-mediated protein–carboxy acid conjugations. A large excess of the amine-containing dyes is usually used for EDC-mediated bioconjugations in concentrated protein solutions at low pH to reduce intra- and inter-protein coupling to lysine residues, a common side reaction.

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iFluor™ Fluorescent Labeling Dyes

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iFluor™ Dyes at-a-glance*

iFluor™ Dye	Alternative to	NH_2-Reactive	SH-Reactive	Labeling Kit
iFluor™ 350	Alexa Fluor® 350, DyLight™ 350, AMCA	1020	1060	1220
iFluor™ 405	Alexa Fluor® 405, DyLight™ 405	1021		
iFluor™ 488	Alexa Fluor® 488, DyLight™ 488, FITC	1023	1062	1255
iFluor™ 514	Alexa Fluor® 514	1024		
iFluor™ 532	Alexa Fluor® 532	1025		
iFluor™ 555	Alexa Fluor® 555, Cy3®, DyLight™ 550	1028	1063	1227
iFluor™ 594	Alexa Fluor® 594, DyLight™ 594, Texas Red®	1029		1230
iFluor™ 633	Alexa Fluor® 633, DyLight™ 633	1030		1260
iFluor™ 647	Alexa Fluor® 647, Cy5®, DyLight™ 650	1031	1065	1235
iFluor™ 680	Alexa Fluor®680, Cy5.5®, DyLight™ 680	1035	1066	1240
iFluor™ 700	Alexa Fluor® 700	1036	1067	1245
iFluor™ 750	Alexa Fluor® 750, Cy7®, DyLight™ 750	1037	1068	1250
iFluor™ 790	Alexa Fluor® 790, DyLight™ 800, IRDye® 800	1368	1366	1265

* Products listed by catalog number

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iFluor™ Dyes, Optimized for Labeling Antibodies and Other Biopolymers

iFluor™ dyes are the products of our recent R&D efforts. AAT Bioquest is rapidly expanding our product lines to meet our customer's 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 our customer to get more research done with less money.

iFluor™ dyes 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 the 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 even significantly outperform Alexa Fluor® labeling dyes on certain antibodies.

Key Features of iFluor™ Dyes

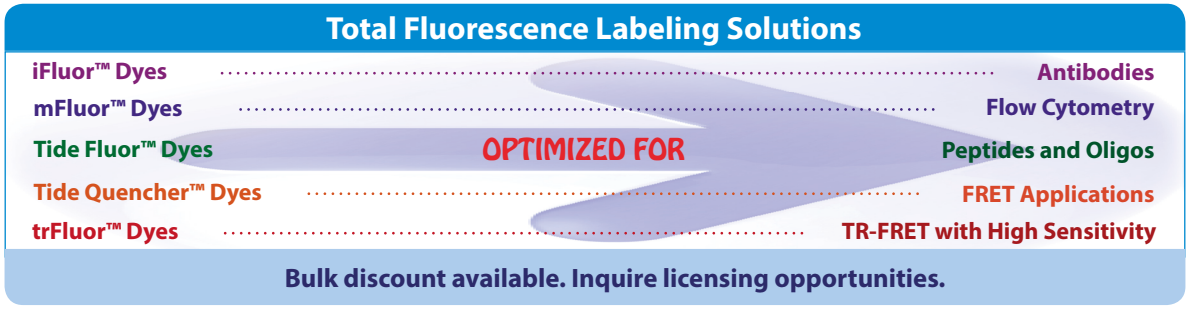
- Have excellent water solubility.
- Available in a variety of reactive forms.
- Available in a variety of distinct fluorescent colors, spanning the full UV-VIS and near infrared range.
- Their conjugates exhibit more intense fluorescence than other spectrally similar conjugates of classic fluorescent 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 with little pH sensitivity.

Table 3.1 Common Fluorescence Excitation Sources Used in Most Fluorescence Instruments

Light Source	Principal Excitation Lines (nm)
Mercury arc lamp	366, 405, 436, 546, 578
Xenon arc lamp	250-1000
Violet diode laser	405
Helium–cadmium laser	325, 442
Argon ion laser	457, 488, 514
Nd:YAG laser	532
Helium–neon laser	543, 594, 633
Yellow diode laser	561
Krypton ion laser	568, 647
Red diode laser	635

Table 3.2 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



iFluor™ 350 Dyes

an Excellent Replacement for AMCA, Alexa Fluor® 350 and DyLight™ 350 Dyes

Quick Summary	
Ex (nm)	345
Em (nm)	442
EC ($cm^{-1}M^{-1}$)	~20,000
$CF_{280\ nm}$*	0.187
$CF_{260\ nm}$**	0.246

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

Although AMCA is the predominant labeling dye for preparing blue fluorescent protein conjugates, AMCA has poor water solubility. iFluor™ 350 dyes are an affordable superior replacement for AMCA. iFluor™ 350 dyes have the spectral properties essentially identical to those of AMCA, DyLight™ 350 and Alexa Fluor® 350 dyes. Protein conjugates prepared with iFluor™ 350 dyes are bright, and their fluorescence is not affected by pH in the physiological range (pH 4-10). The pH insensitivity makes iFluor™ 350 dyes useful for the assays that require extreme pH.

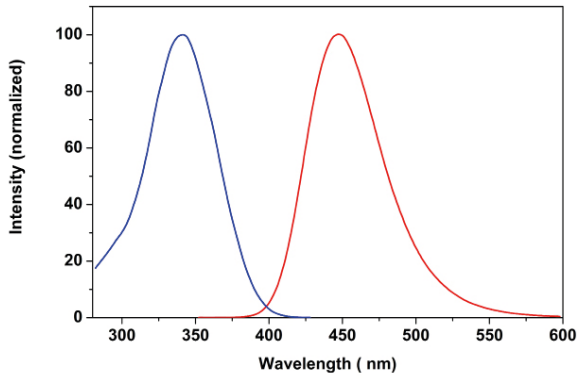


Figure 3.1 The excitation and emission spectra of iFluor™ 350 Goat Anti-Rabbit IgG conjugate (Cat# 16600) in PBS buffer (pH 7.2).

iFluor™ 405 Dyes

an Excellent Replacement for Alexa Fluor® 405 and DyLight™ 405 Dyes

Quick Summary	
Ex (nm)	401
Em (nm)	420
EC ($cm^{-1}M^{-1}$)	~30,000
$CF_{280\ nm}$*	0.697
$CF_{260\ nm}$**	0.229

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

iFluor™ 405 dyes are an excellent replacement for Alexa Fluor® 405 and DyLight™ 405 dyes due to their similar spectral properties. Protein conjugates prepared with iFluor™ 405 dyes are bright, and their fluorescence is not significantly affected by pH in the physiological range (pH 4-10). iFluor™ 405 dyes and conjugates are excellent violet laser reagents for flow cytometry research. Besides iFluor™ 405 violet laser flow cytometry dyes, we also offer multi-color mFluor™ violet laser flow cytometry reagents, including mFluor™ 450, 520 and 550 fluorescent labeling dyes and kits.

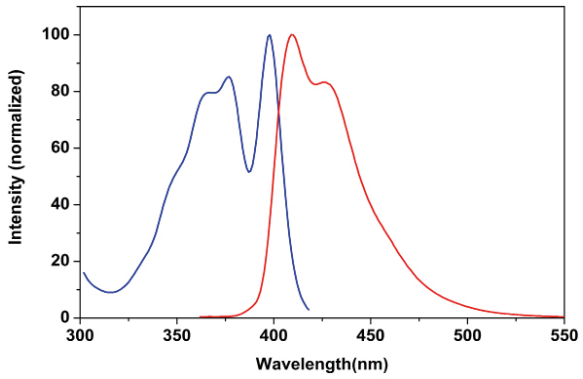


Figure 3.2 The excitation and emission spectra of iFluor™ 405 Goat Anti-Rabbit IgG conjugate (Cat# 16604) in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
16440	200 µg	iFluor™ 350 goat anti-mouse IgG (H+L)	Alexa Fluor® 350, DyLight™ 350
16600	200 µg	iFluor™ 350 goat anti-rabbit IgG (H+L)	Alexa Fluor® 350, DyLight™ 350
1080	1 mg	iFluor™ 350 hydrazide	Alexa Fluor® 350, DyLight™ 350
1060	1 mg	iFluor™ 350 maleimide	Alexa Fluor® 350, DyLight™ 350
16950	200 µg	iFluor™ 350-streptavidin conjugate	Alexa Fluor® 350, DyLight™ 350
1020	1 mg	iFluor™ 350 succinimidyl ester	Alexa Fluor® 350, DyLight™ 350
16444	200 µg	iFluor™ 405 goat anti-mouse IgG (H+L)	Alexa Fluor® 405, DyLight™ 405
16604	200 µg	iFluor™ 405 goat anti-rabbit IgG (H+L)	Alexa Fluor® 405, DyLight™ 405
16952	200 µg	iFluor™ 405-streptavidin conjugate	Alexa Fluor® 405, DyLight™ 405
1021	1 mg	iFluor™ 405 succinimidyl ester	Alexa Fluor® 405, DyLight™ 405
1220	1 kit	ReadiLink™ iFluor™ 350 protein labeling kit	Alexa Fluor® 350, DyLight™ 350

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iFluor™ 488 Dyes
an Excellent Replacement for FITC, DyLight 488™ and Alexa Fluor® 488 Dyes

Quick Summary	
Ex (nm)	491
Em (nm)	514
EC ($cm^{-1}M^{-1}$)	~90,000
$CF_{280\ nm}$*	0.139
$CF_{260\ nm}$**	0.444

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

Although FITC is still the most popular fluorescent labeling dye for preparing green fluorescent bioconjugates, it has certain limitations, such as pH-sensitivity and severe photobleaching for microscope-based fluorescence imaging. Protein conjugates prepared with iFluor™ 488 dyes are far superior for fluorescence imaging applications compared to conjugates of fluorescein derivatives such as FITC. iFluor™ 488 conjugates are significantly brighter than fluorescein conjugates and are much more photostable. Additionally, the fluorescence of iFluor™ 488 is not affected by pH (4-10). The pH insensitivity is a major improvement over fluorescein, which emits its maximum fluorescence only at pH above 9. Compared to Alexa Fluor® 488, iFluor™ 488 of single isomer has much higher purity, making the iFluor™ 488 conjugates demonstrate much higher performance consistency from batch to batch.

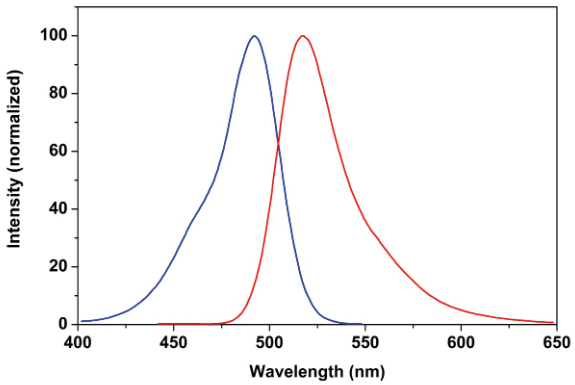

Figure 3.3 The excitation and emission spectra of iFluor™ 488 Goat Anti-Rabbit IgG conjugate (Cat# 16608) in PBS buffer (pH 7.2).

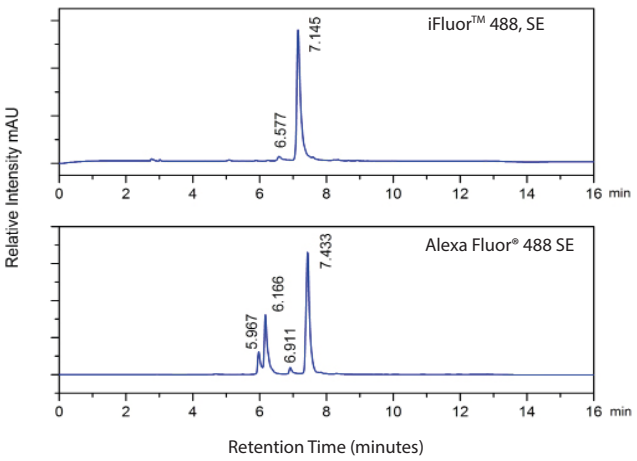

Figure 3.4 HPLC chromatogram comparison of iFluor™ 488 SE (top graph) and Alexa Fluor® 488 SE (bottom graph) indicated iFluor™ 488 SE had much higher purity and lot-to-lot reproducibility.

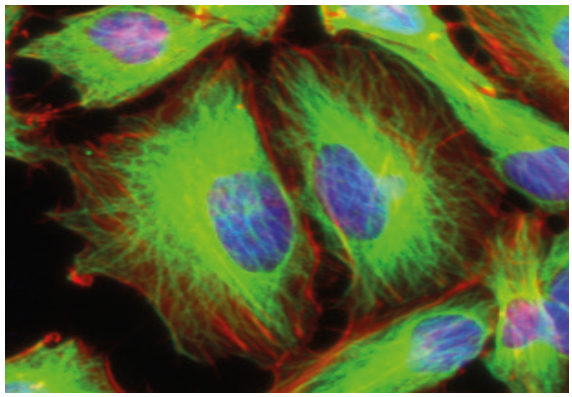

Figure 3.5 Image of HeLa cells. Tublins were stained with mouse anti-tubulin followed with iFluor™ 488 Goat Anti-Mouse IgG (green, Cat# 16448), actin filaments were stained with iFluor™ 555 phalloidin conjugate (red, Cat# 23119), and nuclei were stained with Hoechst 33342 (blue).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
16448	200 µg	iFluor™ 488 goat anti-mouse IgG (H+L)	Alexa Fluor® 488, DyLight™ 488
16608	200 µg	iFluor™ 488 goat anti-rabbit IgG (H+L)	Alexa Fluor® 488, DyLight™ 488
1082	1 mg	iFluor™ 488 hydrazide	Alexa Fluor® 488, DyLight™ 488
1062	1 mg	iFluor™ 488 maleimide	Alexa Fluor® 488, DyLight™ 488
23115	300 tests	iFluor™ 488 phalloidin conjugate	Alexa Fluor® 488, DyLight™ 488
16955	200 µg	iFluor™ 488-streptavidin conjugate	Alexa Fluor® 488, DyLight™ 488
1023	1 mg	iFluor™ 488 succinimidyl ester	Alexa Fluor® 488, DyLight™ 488
1255	1 kit	ReadiLink™ iFluor™ 488 protein labeling kit	Alexa Fluor® 488, DyLight™ 488

iFluor™ 555 Dyes
an Excellent Replacement for Cy3®, DyLight™ 550 and Alexa Fluor® 555 Dyes

Quick Summary	
Ex (nm)	559
Em (nm)	569
EC ($cm^{-1}M^{-1}$)	~150,000
$CF_{280\ nm}$*	0.082
$CF_{260\ nm}$**	0.038

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

Although Cy3® is the preferred dye for preparing orange fluorescent bioconjugates, iFluor™ 555 conjugates are more photostable and brighter. Compared to the spectra of Cy3® conjugates, the spectra of iFluor™ 555 conjugates are slightly red-shifted, resulting in an optimal match to filters designed for Cy3® dyes. The improved photostability of iFluor™ 555 provides researchers with additional time to capture images. iFluor™ 555 dyes are considered an excellent replacement for Cy3®, DyLight™ 550 and Alexa Fluor® 555 dyes.

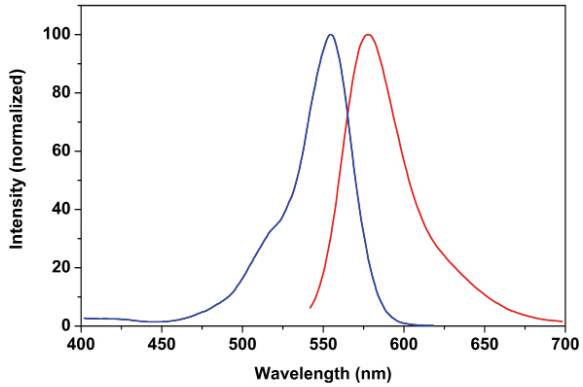

Figure 3.6 The excitation and emission spectra of iFluor™ 555 Goat Anti-Rabbit IgG conjugate (Cat# 16620) in PBS buffer (pH 7.2).

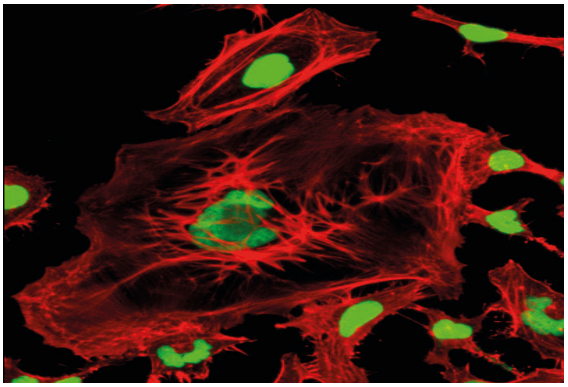

Figure 3.7 Image of HeLa cells. Actin filaments were stained with iFluor™ 555 phalloidin conjugate (red, Cat# 23119), and nuclei were stained with Nuclear Green™ DCS1 (green, Cat# 17550).

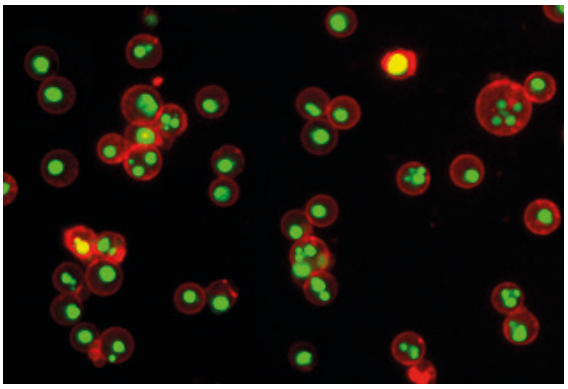

Figure 3.8 Image of apoptotic Jurkat cells. Cells were treated with staurosporine 1 µM for 4 hours, and then stained with Annexin V-iFluor™ 555 conjugate (red, Cat# 20072), and nuclei were stained with Nuclear Green™ DCS1 (green, Cat# 17550).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
20072	100 tests	Annexin V-iFluor™ 555 conjugate	Cy3®, Alexa Fluor® 555, DyLight™ 550
16460	200 µg	iFluor™ 555 goat anti-mouse IgG (H+L)	Cy3®, Alexa Fluor® 555, DyLight™ 550
16540	200 µg	iFluor™ 555 goat anti-mouse IgG (H+L) *Cross Adsorbed*	Cy3®, Alexa Fluor® 555, DyLight™ 550
16620	200 µg	iFluor™ 555 goat anti-rabbit IgG (H+L)	Cy3®, Alexa Fluor® 555, DyLight™ 550
16690	200 µg	iFluor™ 555 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	Cy3®, Alexa Fluor® 555, DyLight™ 550
1083	1 mg	iFluor™ 555 hydrazide	Cy3®, Alexa Fluor® 555, DyLight™ 550
1063	1 mg	iFluor™ 555 maleimide	Cy3®, Alexa Fluor® 555, DyLight™ 550
16959	200 µg	iFluor™ 555-streptavidin conjugate	Cy3®, Alexa Fluor® 555, DyLight™ 550
1028	1 mg	iFluor™ 555 succinimidyl ester	Cy3®, Alexa Fluor® 555, DyLight™ 550
23119	300 tests	Phalloidin-iFluor™ 555 conjugate	Cy3®, Alexa Fluor® 555, DyLight™ 550
1227	1 kit	ReadiLink™ iFluor™ 555 protein labeling kit	Cy3®, Alexa Fluor® 555, DyLight™ 550

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info@biomol.de · www.biomol.de

iFluor™ 594 Dyes

an Excellent Replacement for Texas Red®, DyLight™ 594 and Alexa Fluor® 594 Dyes

Quick Summary	
Ex (nm)	592
Em (nm)	614
EC ($cm^{-1}M^{-1}$)	~90,000
$CF_{280\ nm}$*	0.187
$CF_{260\ nm}$**	0.234

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

iFluor™ 594 has spectral characteristics similar to those of Texas Red®, DyLight™ 594 and Alexa Fluor® 594 with excitation and emission wavelength at ~592/614 nm when conjugated to proteins. iFluor™ 594 dyes have superior labeling performance and better stability than Texas Red®. Our iFluor™ 594 conjugated streptavidin provides high fluorescence intensity and low background as validated in immunofluorescence staining of mammalian cells. Biomolecules conjugated to iFluor™ 594 exhibit little spectral overlap with green-fluorescent conjugates, and can be efficiently excited by 568 nm line of Ar-Kr laser and by the 594 nm line of orange He-Ne laser. The minimal spectral overlap makes iFluor™ 594 an ideal second color in combination with a green color such as GFP, FITC, Alexa Fluor® 488 or iFluor™ 488. Our in-house research indicated that the iFluor™ 594-RPE conjugates demonstrate better FRET than Alexa Fluor® 594-RPE.

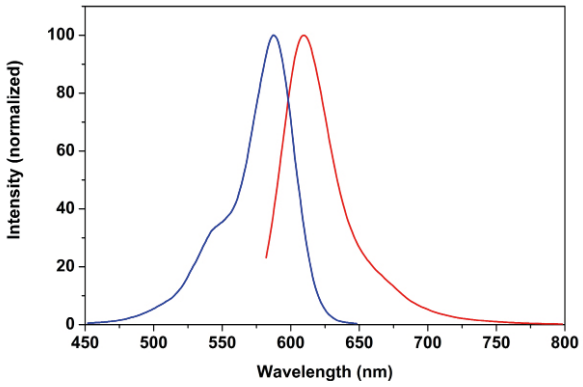

Figure 3.9 The excitation and emission spectra of iFluor™ 594 Goat Anti-Rabbit IgG conjugate (Cat# 16628) in PBS buffer (pH 7.2).

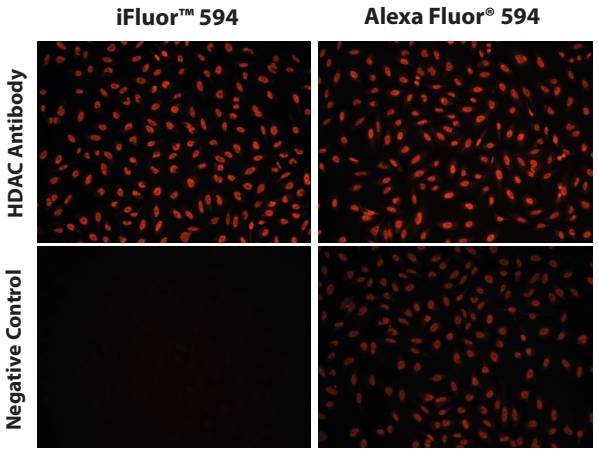

Figure 3.10 iFluor™ 594 gave much higher conjugation yield than Alexa Fluor® 594. HeLa cells were stained with Rabbit HDAC antibody, and then followed with iFluor™ 594 Goat Anti-Rabbit IgG conjugate and Alexa Fluor® 594 Goat Anti-Rabbit IgG conjugate respectively under the same conditions. The iFluor™ 594 Goat Anti-Rabbit IgG conjugate (left panel) demonstrated much lower staining background than the corresponding Alexa Fluor® 594 (right panel).

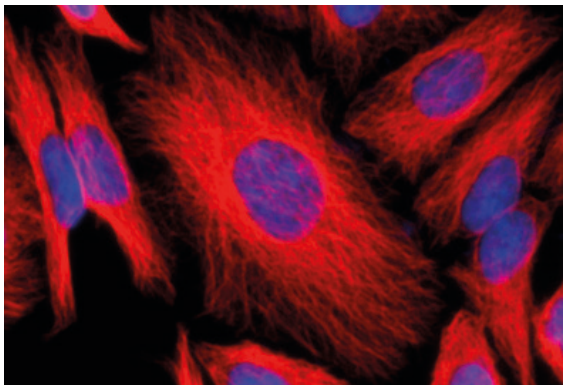

Figure 3.11 HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 594 Goat Anti-Mouse IgG (red, Cat# 16468), and nuclei were stained with Hoechst 33342 (blue, Cat# 17530).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
20073	100 tests	Annexin V-iFluor™ 594 conjugate	Texas Red®, Alexa Fluor® 594, DyLight™ 594
16468	200 µg	iFluor™ 594 goat anti-mouse IgG (H+L)	Texas Red®, Alexa Fluor® 594, DyLight™ 594
16628	200 µg	iFluor™ 594 goat anti-rabbit IgG (H+L)	Texas Red®, Alexa Fluor® 594, DyLight™ 594
2577	1 mg	iFluor™ 594-RPE tandem	Texas Red®, Alexa Fluor® 594, DyLight™ 594
16962	200 µg	iFluor™ 594-streptavidin conjugate	Texas Red®, Alexa Fluor® 594, DyLight™ 594
1029	1 mg	iFluor™ 594 succinimidyl est	Texas Red®, Alexa Fluor® 594, DyLight™ 594
1230	1 kit	ReadiLink™ iFluor™ 594 protein labeling kit	Texas Red®, Alexa Fluor® 594, DyLight™ 594

iFluor™ 633 Dyes
an Excellent Replacement for DyLight™ 633 and Alexa Fluor® 633 Dyes

Quick Summary	
Ex (nm)	638
Em (nm)	655
EC ($cm^{-1}M^{-1}$)	~250,000
$CF_{280\ nm}$*	0.045
$CF_{260\ nm}$**	0.062

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

iFluor™ 633 dyes are spectrally similar to Alexa Fluor® 633 and DyLight™ 633 dyes. Fluorescence emission of iFluor™ 633 dyes is well separated from that of other commonly used red fluorophores, such as TAMRA, Texas Red®, Alexa Fluor® 594, iFluor™ 594 and R-phycoerythrin. iFluor™ 633 dyes can be well excited by the 633 nm red laser in flow cytometers, giving a deep red emission. Compared to Alexa Fluor® 633, the extinction coefficient of iFluor™ 633 is much higher (~250,000 $cm^{-1}M^{-1}$).

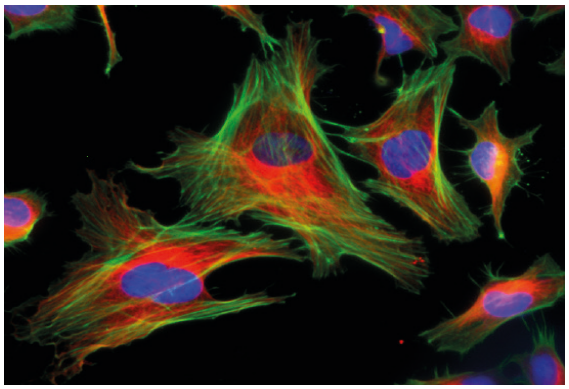

Figure 3.13 HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 633 Goat Anti-Mouse IgG (red, Cat# 16478), actin filaments were stained with iFluor™ 488 phalloidin conjugate (green, Cat# 23115), and nuclei were stained with Hoechst 33342 (blue, Cat# 17530).

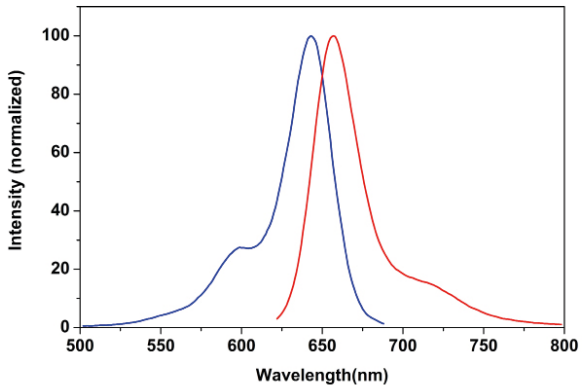

Figure 3.12 The excitation and emission spectra of iFluor™ 633 Goat Anti-Rabbit IgG conjugate (Cat# 16638) in PBS buffer (pH 7.2).

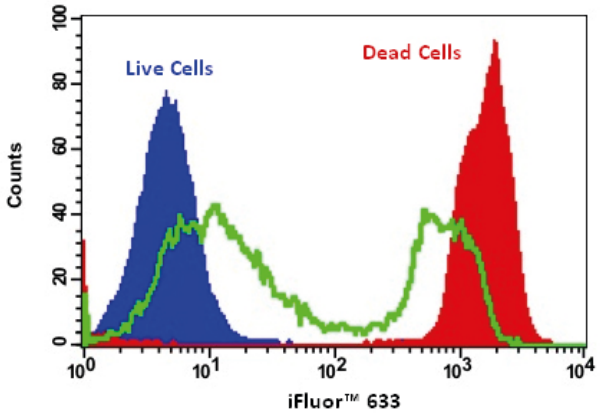

Figure 3.14 Detection of Jurkat cell viability with iFluor™ 633 SE (Cat# 1030). Blue: live cells; Red: heat-treated; Green: staurosporine treated. The live cell population was easily distinguished from the dead cell population.

Product Ordering Information

Cat #	Size	Product Name	Alternative to
16478	200 µg	iFluor™ 633 goat anti-mouse IgG (H+L)	Alexa Fluor® 633, DyLight™ 633
16558	200 µg	iFluor™ 633 goat anti-mouse IgG (H+L) *Cross Adsorbed*	Alexa Fluor® 633, DyLight™ 633
16638	200 µg	iFluor™ 633 goat anti-rabbit IgG (H+L)	Alexa Fluor® 633, DyLight™ 633
16704	200 µg	iFluor™ 633 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	Alexa Fluor® 633, DyLight™ 633
16965	200 µg	iFluor™ 633-streptavidin conjugate	Alexa Fluor® 633, DyLight™ 633
1030	1 mg	iFluor™ 633 succinimidyl ester	Alexa Fluor® 633, DyLight™ 633
23125	300 tests	Phalloidin-iFluor™ 633 conjugate	Alexa Fluor® 633, DyLight™ 633
1260	1 kit	ReadiLink™ iFluor™ 633 protein labeling kit	Alexa Fluor® 633, DyLight™ 633

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info@biomol.de · www.biomol.de

iFluor™ 647 Dyes
an Excellent Replacement for Cy5®, DyLight™ 650 and Alexa Fluor® 647 Dyes

Quick Summary	
Ex (nm)	654
Em (nm)	674
EC ($cm^{-1}M^{-1}$)	~250,000
$CF_{280\ nm}$*	0.039
$CF_{260\ nm}$**	0.046

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

The slight red shift in absorption spectrum makes iFluor™ 647 dyes an optimal match to filters designed for Cy5® dyes. In side-by-side comparison of antibody conjugates of iFluor™ 647 dyes and Cy5® conjugates, the total fluorescence of iFluor™ 647 labeled secondary antibodies is significantly higher than that of Cy5® conjugates. Unlike Cy5® dyes, iFluor™ 647 dyes have very little change in absorption or fluorescence spectra when conjugated to most proteins and nucleic acids, thus yielding greater total fluorescence at the same degree of substitution. iFluor™ 647 dyes are considered an excellent replacement for Cy5®, Alexa Fluor® 647 and DyLight™ 650 dyes.

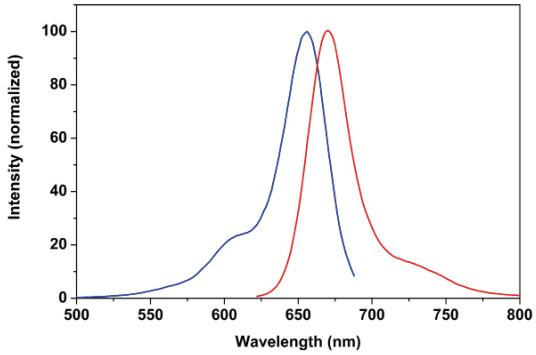

Figure 3.15 The excitation and emission spectra of iFluor™ 647 Goat Anti-Rabbit IgG conjugation (Cat# 16642) in PBS buffer (pH 7.2) .

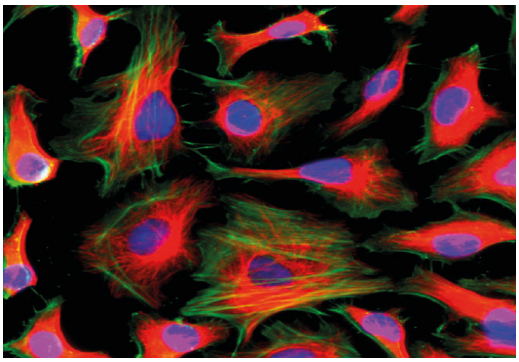

Figure 3.16 HeLa cells were stained with mouse anti-tubulin followed with iFluor™ 647 goat anti-mouse IgG (red, Cat# 16482), actin filaments were stained with iFluor™ 488 phalloidin conjugate (green), and nuclei were stained with Hoechst 33342 (blue, Cat# 17530).

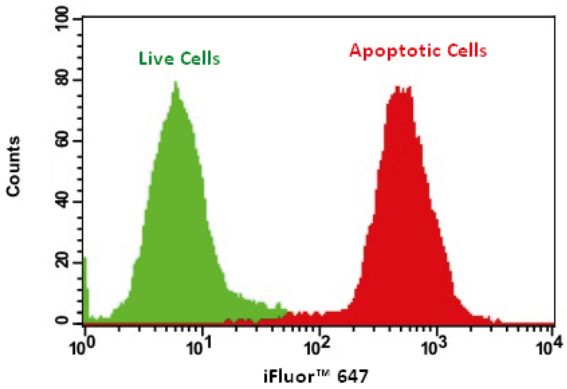

Figure 3.17 Detection of phosphatidylserine binding activity in Jurkat cells. Jurkat cells were stained with Annexin V-iFluor™ 647 conjugate (Cat# 20074) for 30 minutes. Green: untreated live cells; Red: apoptotic cells treated with 1 µM staurosporine for 4 hours.

Product Ordering Information

Cat #	Size	Product Name	Alternative to
1090	1 mg	iFluor™ 647 alkyne	Cy5®, DyLight™ 650, Alexa Fluor® 647
1091	1 mg	iFluor™ 647 azide	Cy5®, DyLight™ 650, Alexa Fluor® 647
16482	200 µg	iFluor™ 647 goat anti-mouse IgG (H+L)	Cy5®, DyLight™ 650, Alexa Fluor® 647
16562	200 µg	iFluor™ 647 goat anti-mouse IgG (H+L) *Cross Adsorbed*	Cy5®, DyLight™ 650, Alexa Fluor® 647
16642	200 µg	iFluor™ 647 goat anti-rabbit IgG (H+L)	Cy5®, DyLight™ 650, Alexa Fluor® 647
16710	200 µg	iFluor™ 647 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	Cy5®, DyLight™ 650, Alexa Fluor® 647
1085	1 mg	iFluor™ 647 hydrazide	Cy5®, DyLight™ 650, Alexa Fluor® 647
1065	1 mg	iFluor™ 647 maleimide	Cy5®, DyLight™ 650, Alexa Fluor® 647
1031	1 mg	iFluor™ 647 succinimidyl ester	Cy5®, DyLight™ 650, Alexa Fluor® 647
1235	1 kit	ReadiLink™ iFluor™ 647 protein labeling kit	Cy5®, DyLight™ 650, Alexa Fluor® 647
16906	100 µg	RPE-iFluor™ 647-streptavidin conjugate	Cy5®, DyLight™ 650, Alexa Fluor® 647
2577	1 mg	RPE-iFluor™ 647 tandem	Cy5®, DyLight™ 650, Alexa Fluor® 647

iFluor™ 680 Dyes

an Excellent Replacement for Cy5.5®, IRDye® 700 and Alexa Fluor® 680 Dyes

Quick Summary	
Ex (nm)	682
Em (nm)	701
EC ($cm^{-1}M^{-1}$)	~250,000
$CF_{280\ nm}$*	0.111
$CF_{260\ nm}$**	0.121

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

iFluor™ 680 dyes are spectrally similar to Cy5.5®, IRDye® 700, Alexa Fluor® 680 and DyLight™ 680 dyes. Fluorescence emission of iFluor™ 680 dyes is well separated from that of other commonly used red fluorophores, such as TAMRA, R-phycoerythrin, iFluor™ 594, 633 and 647 dyes, making it ideal for three and four-color labeling. iFluor™ 688 dyes can be effectively excited by the 633 nm red laser in flow cytometers, giving a near infrared emission. iFluor™ 680 dyes are also excellent acceptor dyes for allophycocyanin (APC), further facilitating multicolor flow cytometry analysis. We offer iFluor™ 688-APC tandem conjugates for flow cytometric applications.

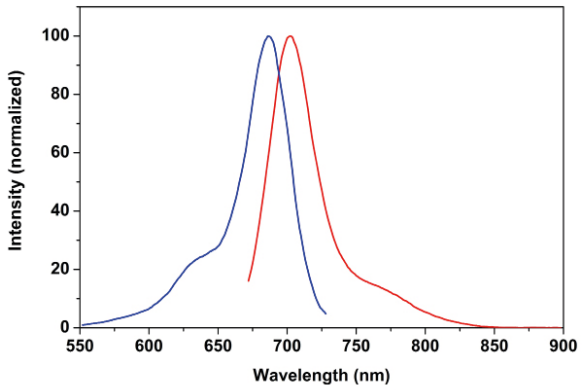

Figure 3.18 The excitation and emission spectra of iFluor™ 680 Goat Anti-Rabbit IgG conjugate (Cat# 16646) in PBS buffer (pH 7.2).

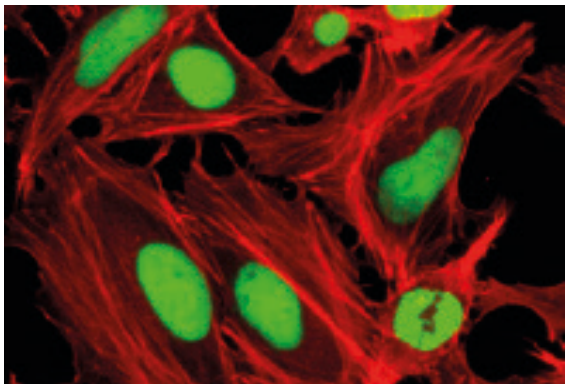

Figure 3.19 Image of HeLa cells. Actin filaments were stained with iFluor™ 680 phalloidin conjugate (red, Cat# 23128), and nuclei were stained with Nuclear Green™ DCS1 (green, Cat#17550).

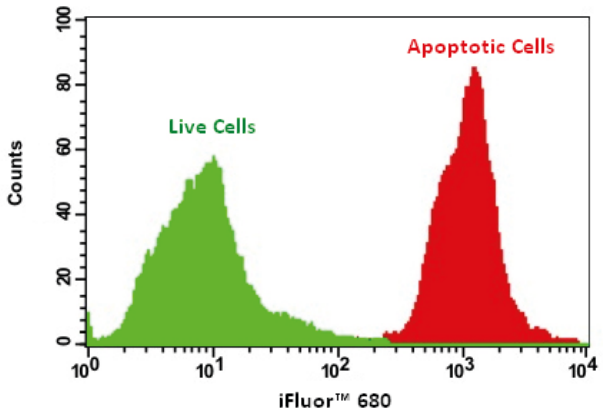

Figure 3.20 Detection of phosphatidylserine binding activity in Jurkat cells. Jurkat cells were stained with Annexin V-iFluor™ 680 conjugate (Cat# 20075) for 30 minutes. Green: untreated live cells; Red: apoptotic cells treated with 1 µM staurosporine for 4 hours.

Product Ordering Information

Cat #	Size	Product Name	Alternative to
20075	100 tests	Annexin V-iFluor™ 680 conjugate	Cy5.5®, IRDye® 700, Alexa Fluor® 680
16486	200 µg	iFluor™ 680 goat anti-mouse IgG (H+L)	Cy5.5®, IRDye® 700, Alexa Fluor® 680
16566	200 µg	iFluor™ 680 goat anti-mouse IgG (H+L) *Cross Adsorbed*	Cy5.5®, IRDye® 700, Alexa Fluor® 680
16646	200 µg	iFluor™ 680 goat anti-rabbit IgG (H+L)	Cy5.5®, IRDye® 700, Alexa Fluor® 680
16712	200 µg	iFluor™ 680 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	Cy5.5®, IRDye® 700, Alexa Fluor® 680
1066	1 mg	iFluor™ 680 maleimide	Cy5.5®, IRDye® 700, Alexa Fluor® 680
16968	200 µg	iFluor™ 680-streptavidin conjugate	Cy5.5®, IRDye® 700, Alexa Fluor® 680
1035	1 mg	iFluor™ 680 succinimidyl ester	Cy5.5®, IRDye® 700, Alexa Fluor® 680
23128	300 tests	Phalloidin-iFluor™ 680 conjugate	Cy5.5®, IRDye® 700, Alexa Fluor® 680
1240	1 kit	ReadiLink™ iFluor™ 680 protein labeling kit	Cy5.5®, IRDye® 700, Alexa Fluor® 680

Tel.: 0800-246 66 51 · Fax: 0800-246 66 52
info@biomol.de · www.biomol.de

iFluor™ 700 Dyes
an Excellent Replacement for Alexa Fluor® 700 Dyes

Quick Summary	
Ex (nm)	693
Em (nm)	713
EC ($cm^{-1}M^{-1}$)	~250,000
$CF_{280\ nm}$*	0.164
$CF_{260\ nm}$**	0.188

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

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

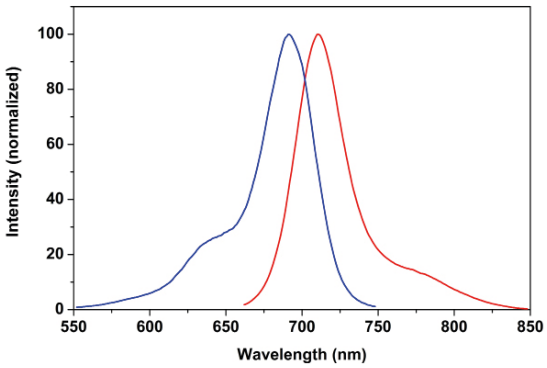

Figure 3.21 The excitation and emission spectra of iFluor™ 700 Goat Anti-Rabbit IgG conjugate (Cat# 16652) in PBS buffer (pH 7.2).

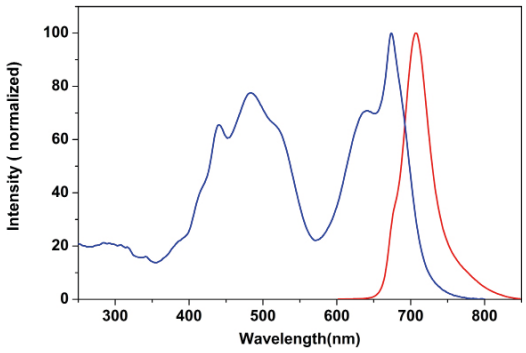

Figure 3.22 The excitation and emission spectra of PerCP-iFluor™ 700 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

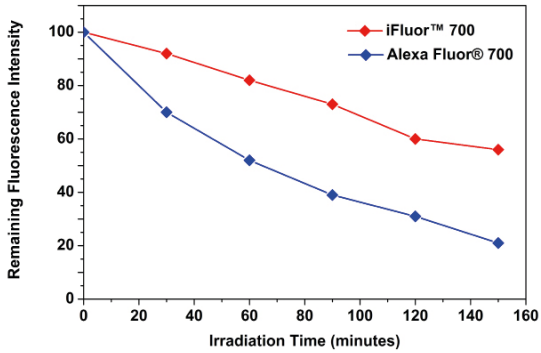

Figure 3.23 Photostability comparison of APC-iFluor™ 700 tandem with the spectrally equivalent APC-Alexa Fluor® 700 tandem in PBS buffer (pH 7.2). Both iFluor™ 700 and Alexa Fluor® tandems were irradiated with 200 W lamp in PBS (pH 7.2), where all of the dyes received the same amount of irradiation as determined by photometric measurements. As shown above, iFluor™ 700 tandem exhibited much higher photostability than the corresponding Alexa Fluor® 700-APC tandem.

Product Ordering Information

Cat #	Size	Product Name	Alternative to
20077	100 tests	Annexin V-iFluor™ 700 conjugate	Alexa Fluor® 700
2570	1 mg	APC-iFluor™ 700 tandem	Alexa Fluor® 700
16494	200 µg	iFluor™ 700 goat anti-mouse IgG (H+L)	Alexa Fluor® 700
16574	200 µg	iFluor™ 700 goat anti-mouse IgG (H+L) *Cross Adsorbed*	Alexa Fluor® 700
16652	200 µg	iFluor™ 700 goat anti-rabbit IgG (H+L)	Alexa Fluor® 700
16714	200 µg	iFluor™ 700 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	Alexa Fluor® 700
1087	1 mg	iFluor™ 700 hydrazide	Alexa Fluor® 700
1067	1 mg	iFluor™ 700 maleimide	Alexa Fluor® 700
16970	200 µg	iFluor™ 700-streptavidin conjugate	Alexa Fluor® 700
1036	1 mg	iFluor™ 700 succinimidyl ester	Alexa Fluor® 700
1245	1 kit	ReadiLink™ iFluor™ 700 protein labeling kit	Alexa Fluor® 700

iFluor™ 750 Dyes

an Excellent Replacement for Cy7®, DyLight™ 755 and Alexa Fluor® 750 Dyes

Quick Summary	
Ex (nm)	753
Em (nm)	779
EC ($cm^{-1}M^{-1}$)	~250,000
$CF_{280\ nm}$*	0.114
$CF_{260\ nm}$**	0.114

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

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

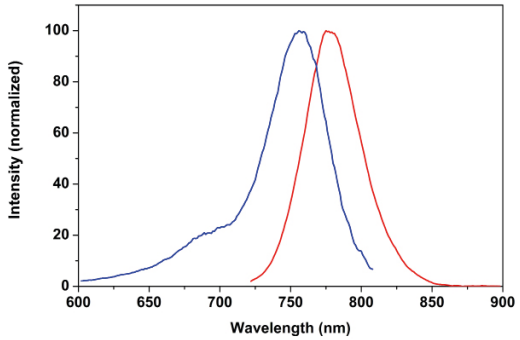

Figure 3.24 The excitation and emission spectra of iFluor™ 750 Goat Anti-Rabbit IgG conjugate (Cat# 16660) in PBS buffer (pH 7.2).

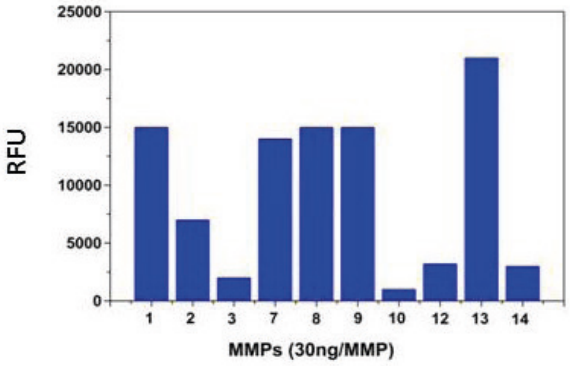

Figure 3.25 Detection of MMPs using MMP Infrared™ substrate, an infrared FRET substrate consisting of iFluor™ 750 (donor) and Tide Quencher™ 7 (acceptor). The APMA-activated MMPs, 30 ng each, were mixed with MMP Infrared™ substrate (10 µM). The fluorescence signal was monitored at 1 hour upon starting the enzyme reaction using a filter set of Ex/Em = 740/780 nm. The MMP Infrared™ substrate can detect the activity of sub-nanogram of all MMPs (n=3). This novel infrared FRET substrate can also be used for *in vivo* detection of MMP activities.

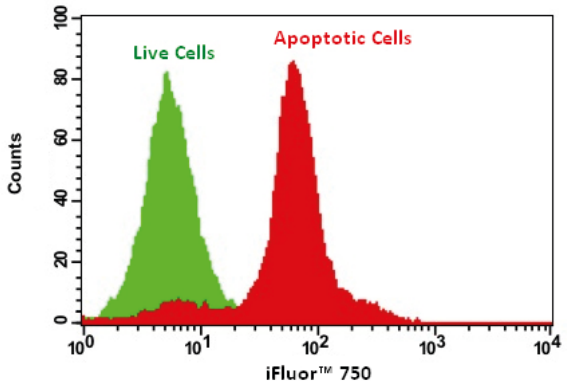

Figure 3.26 Detection of phosphatidylserine binding activity in Jurkat cells. Jurkat cells were stained with Annexin V-iFluor™ 750 conjugate (Cat# 20076) for 30 minutes. Blue: untreated live cells; Red: apoptotic cells treated with 1 µM staurosporine for 4 hours.

Product Ordering Information

Cat #	Size	Product Name	Alternative to
20076	100 tests	Annexin V-iFluor™ 750 conjugate	Cy7®, DyLight™ 755, Alexa Fluor® 750
2571	1 mg	APC-iFluor™ 750 tandem	Cy7®, DyLight™ 755, Alexa Fluor® 750
16506	200 µg	iFluor™ 750 goat anti-mouse IgG (H+L)	Cy7®, DyLight™ 755, Alexa Fluor® 750
16586	200 µg	iFluor™ 750 goat anti-mouse IgG (H+L) *Cross Adsorbed*	Cy7®, DyLight™ 755, Alexa Fluor® 750
16660	200 µg	iFluor™ 750 goat anti-rabbit IgG (H+L)	Cy7®, DyLight™ 755, Alexa Fluor® 750
16720	200 µg	iFluor™ 750 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	Cy7®, DyLight™ 755, Alexa Fluor® 750
1068	1 mg	iFluor™ 750 maleimide	Cy7®, DyLight™ 755, Alexa Fluor® 750
16973	200 µg	iFluor™ 750-streptavidin conjugate	Cy7®, DyLight™ 755, Alexa Fluor® 750
1037	1 mg	iFluor™ 750 succinimidyl ester	Cy7®, DyLight™ 755, Alexa Fluor® 750
1250	1 kit	ReadiLink™ iFluor™ 750 protein labeling kit	Cy7®, DyLight™ 755, Alexa Fluor® 750
2578	1 mg	RPE-iFluor™ 750 tandem	Cy7®, DyLight™ 755, Alexa Fluor® 750

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iFluor™ 790 Dyes

an Excellent Replacement for IRDye® 800 and Alexa Fluor® 790 Dyes

Quick Summary	
Ex (nm)	782
Em (nm)	811
EC ($cm^{-1}M^{-1}$)	~250,000
$CF_{280\ nm}$*	0.224
$CF_{260\ nm}$**	0.251

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

Spectrally similar to IRDye®800 and Alexa Fluor® 790 dyes, iFluor™ 790 dyes are the longest-wavelength iFluor™ dyes that AAT Bioquest currently offers. Its fluorescence emission maximum around 810 nm is well separated from commonly used far-red fluorophores, including iFluor™ 647, iFluor™ 680 or allophycocyanin (APC), facilitating the rapidly growing NIR biological analysis. iFluor™ 790 is one of the brightest NIR fluorescent labeling dyes with fluorescence quantum yield close to 0.1. Its conjugates have been successfully used for NIR fluorescent probes-based *in vivo* imaging analysis.

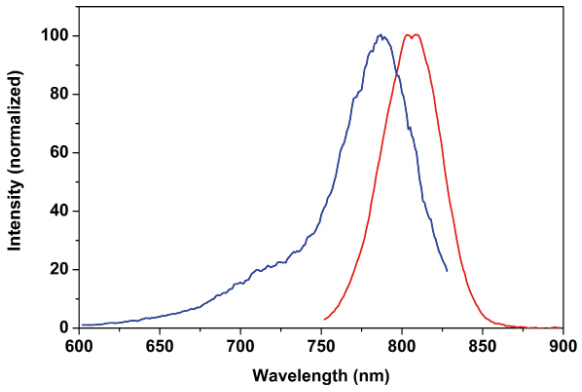


Figure 3.27 The excitation and emission spectra of iFluor™ 790 Goat Anti-Rabbit IgG conjugate (Cat# 16661) in PBS buffer (pH 7.2).

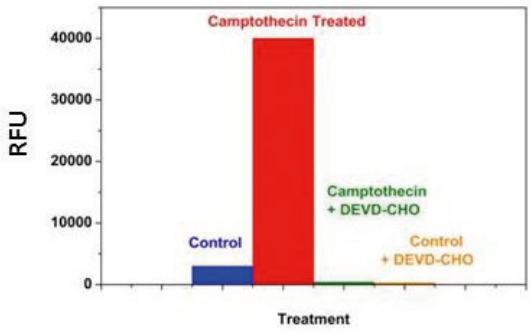


Figure 3.28 Detection of caspase-3 activity in Jurkat cells with Z-KKK(iFluor™ 790)RKVGKDEVDKKC(TQ-8). Jurkat cells were treated with or without 20 mM camptothecin for 5 hours, and/or 5 mM of the caspase inhibitor AC-DEVD-CHO for 10 min. The caspase-3 assay solution was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 780/810 nm. This iFluor™ 790-based NIR fluorogenic FRET substrate was also used for *in vivo* apoptosis detection.

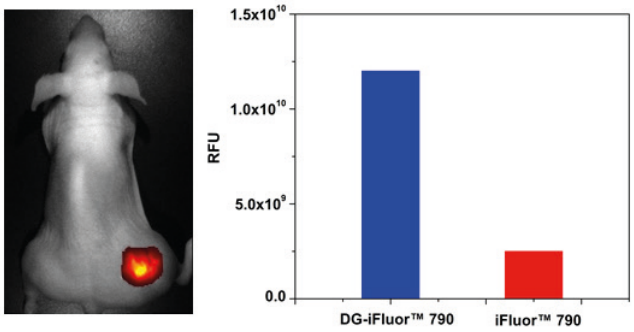


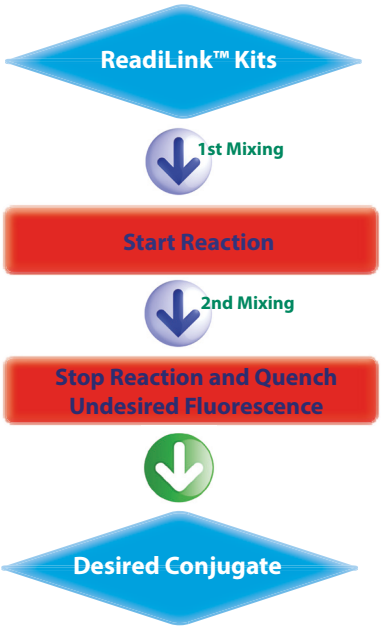
Figure 3.29 Mice with tumor were intravenously injected with 10 nmol of 2-Deoxyaminoglucose (DG)-iFluor™ 790 conjugate or control iFluor™ 790 acid and imaged at the 6th hour. Mouse injected with the DG-iFluor™ 790 showed specific accumulation of the iFluor™ 790 probe to the tumor site.

Product Ordering Information

Cat #	Size	Product Name	Alternative to
1360	5 mg	iFluor™ 790 acid	IRDye® 800, Alexa Fluor® 790
1362	1 mg	iFluor™ 790 amine	IRDye® 800, Alexa Fluor® 790
16507	200 µg	iFluor™ 790 goat anti-mouse IgG (H+L)	IRDye® 800, Alexa Fluor® 790
16587	200 µg	iFluor™ 790 goat anti-mouse IgG (H+L) *Cross Adsorbed*	IRDye® 800, Alexa Fluor® 790
16661	200 µg	iFluor™ 790 goat anti-rabbit IgG (H+L)	IRDye® 800, Alexa Fluor® 790
16721	200 µg	iFluor™ 790 goat anti-rabbit IgG (H+L) *Cross Adsorbed*	IRDye® 800, Alexa Fluor® 790
1366	1 mg	iFluor™ 790 maleimide	IRDye® 800, Alexa Fluor® 790
36801	1 mg	iFluor™ 790 RGD conjugate	IRDye® 800, Alexa Fluor® 790
1368	1 mg	iFluor™ 790 succinimidyl ester	IRDye® 800, Alexa Fluor® 790
23131	300 tests	Phalloidin-iFluor™ 790 conjugate	IRDye® 800, Alexa Fluor® 790
1265	1 kit	ReadiLink™ iFluor™ 790 protein labeling kit	IRDye® 800, Alexa Fluor® 790

ReadiLink™ iFluor™ Protein Labeling Kits

ReadiLink™ iFluor™ Protein Labeling Kits provide a convenient way to label proteins using the stable reactive form of the iFluor™ dyes. The reactive iFluor™ dyes show good reactivity and selectivity with the aliphatic amines of proteins and forms a carboxamide bond, which is identical to, and is as stable as the natural peptide bond. iFluor™-protein 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™-protein conjugates. ReadiLink™ Kits only require two simple mixing steps to produce the desired conjugates for flow cytometry and fluorescence imaging applications.



Key Features of ReadiLink™ Kits :

- Complete, all the components provided in the kits.
- Simple, only two mixing steps required.
- Rapid, less than 10 minutes hands-on time.

The Best Custom Bioconjugation Service

Together We Shine℠

- Biotinylation of proteins and antibodies with ReadiView™ biotin.
- Labeling proteins and antibodies with iFluor™ , mFluor™, trFluor™ and other dyes.
- Labeling proteins and antibodies with APC, RPE and PerCP.
- Labeling proteins and antibodies with enzymes.
- Lableing small bioactive compounds.

24 Hours Turnaround*

* APC and RPE custom conjugation services require 3 working days.

Product Ordering Information

Cat #	Size	Product Name	Alternative to
1220	1 kit	ReadiLink™ iFluor™ 350 protein labeling kit	Lightning-Link® dye labeling kits
1255	1 kit	ReadiLink™ iFluor™ 488 protein labeling kit	Lightning-Link® dye labeling kits
1227	1 kit	ReadiLink™ iFluor™ 555 protein labeling kit	Lightning-Link® dye labeling kits
1230	1 kit	ReadiLink™ iFluor™ 594 protein labeling kit	Lightning-Link® dye labeling kits
1260	1 kit	ReadiLink™ iFluor™ 633 protein labeling kit	Lightning-Link® dye labeling kits
1235	1 kit	ReadiLink™ iFluor™ 647 protein labeling kit	Lightning-Link® dye labeling kits
1240	1 kit	ReadiLink™ iFluor™ 680 protein labeling kit	Lightning-Link® dye labeling kits
1245	1 kit	ReadiLink™ iFluor™ 700 protein labeling kit	Lightning-Link® dye labeling kits
1250	1 kit	ReadiLink™ iFluor™ 750 protein labeling kit	Lightning-Link® dye labeling kits
1265	1 kit	ReadiLink™ iFluor™ 790 protein labeling kit	Lightning-Link® dye labeling kits

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mFluor™ Fluorescent Labeling Dyes

4

mFluor™ Labeling Dyes at-a-glance

Emission Color \ Excitation Laser	Violet (405 nm)	Blue (488 nm)	Green (532 nm)	Yellow (561 nm)	Red (633 nm)
Blue	**mFluor™ 450**				
Green	**mFluor™ 510**				
Orange	**mFluor™ 540**	**mFluor™ Blue 570**			
Red			**mFluor™ Green 620**	**mFluor™ Yellow 630**	
Far Red					**mFluor™ Red 700**
Infrared					**mFluor™ Red 780**

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mFluor™ Fluorescent Labeing Dyes

Optimized for Flow Cytometry Applications

mFluor™ dyes are the products of our recent R&D efforts. AAT Bioquest is rapidly expanding our product lines to meet your constantly changing research needs. We have been developing dyes to solve various limitations with the existing fluorescent labeling reagents. 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.

Table 4.1 Common Fluorescence Excitation Sources Used in Most Flow Cytometers

Light Source	Principal Excitation Lines (nm)
Violet Laser	405
Blue Laser	488
Green Laser	532
Yellow-Green Laser	561
Red Laser	633

Table 4.2 mFluor™ Dyes

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

Key Features of mFluor™ Dyes:

- mFluor™ dyes are available in a variety of reactive forms.
- mFluor™ dyes are much easier for conjugation, giving much higher conjugation yields than tandems.
- mFluor™ conjugates are maximally excited by one of the major light sources used in flow cytometers.
- mFluor™ dyes are much more photostable than the phycobiliprotein tandems.
- mFluor™ dyes and their conjugates are available in a few distinct fluorescence colors.
- mFluor™ dyes are robust and highly fluorescent over a broad pH range with little pH sensitivity.

Custom Labeling Service

Together We Shine℠

Labels Available

Biotin, APC, RPE, PerCP, FITC, Texas Red®
iFluor™ dyes for labeling antibodies
mFluor™ dyes for flow cytometry applications
Tide Fluor™ and Tide Quencher™ dyes for FRET, oligos and peptides
trFluor™ dyes for TR-FRET applications

Targets Conjugatable

Antibodies, proteins, peptides, carbohydrates, lipids, oligos, nucleic acids and small drug molecules

24 Hours Turnaround*

* APC and RPE custom conjugation services require 3 working days.

mFluor™ Violet 450 Dyes
an Excellent Replacement for Pacific Blue® Dyes

Quick Summary	
Ex (nm)	403
Em (nm)	454
EC ($cm^{-1}M^{-1}$)	~35,000
$CF_{280\ nm}$*	0.238

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

mFluor™ Violet 450 dyes are an excellent replacement for Pacific Blue® dyes since they have the spectral properties equivalent to those of Pacific Blue® dyes. mFluor™ Violet 450 dyes are much more water-soluble than Pacific Blue® dyes. Our in-house research indicated that the protein conjugates prepared with mFluor™ Violet 450 are brighter than Pacific Blue®. mFluor™ Violet 450 dyes and conjugates are excellent violet laser reagents for flow cytometry research. All the mFluor™ Violet 450 conjugates are compatible with the filter set of 450/50 nm.

mFluor™ Violet 510 Dyes
an Excellent Alternative to AmCyan

Quick Summary	
Ex (nm)	414
Em (nm)	508
EC ($cm^{-1}M^{-1}$)	~25,000
$CF_{280\ nm}$*	0.781

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

mFluor™ Violet 510 dyes are an excellent alternative to AmCyan since they have the spectral properties equivalent to those of AmCyan. mFluor™ Violet 510 dyes are water-soluble, and the protein conjugates prepared with mFluor™ Violet 510 dyes are well excited at 405 nm to give green fluorescence (compatible with FITC filter). mFluor™ Violet 510 dyes and conjugates are excellent violet laser reagents for flow cytometry research. All the mFluor™ Violet 510 conjugates are compatible with the filter set of 525/20 nm.

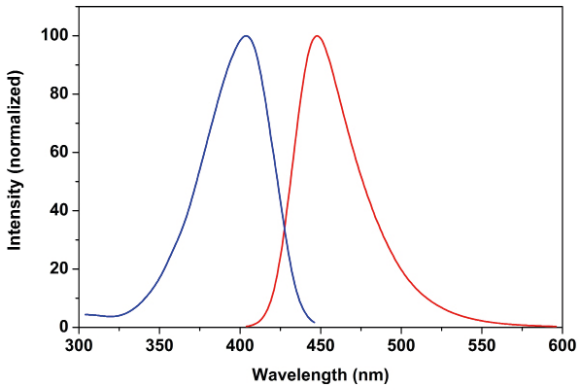

Figure 4.1 The excitation and emission spectra of mFluor™ Violet 450 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

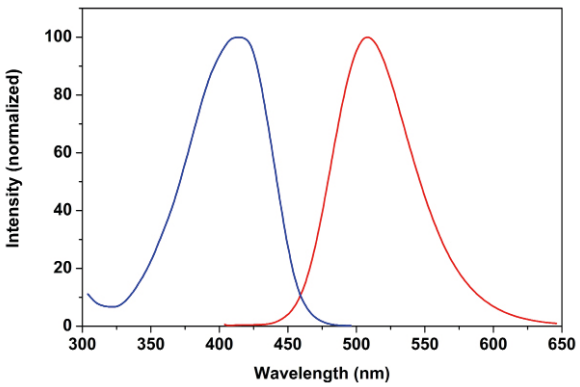

Figure 4.2 The excitation and emission spectra of mFluor™ Violet 510 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
20080	100 tests	Annexin V-mFluor™ Violet 450 conjugate	Pacific Blue®
20081	100 tests	Annexin V-mFluor™ Violet 510 conjugate	AmCyan
1150	1 mg	mFluor™ Violet 450 SE	Pacific Blue®
16930	100 µg	mFluor™ Violet 450-streptavidin conjugate	Pacific Blue®
1151	1 mg	mFluor™ Violet 510 SE	AmCyan
16931	100 µg	mFluor™ Violet 510-streptavidin conjugate	AmCyan
1100	1 kit	ReadiLink™ mFluor™ Violet 450 protein labeling kit	Pacific Blue®
1110	1 kit	ReadiLink™ mFluor™ Violet 510 protein labeling kit	AmCyan

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mFluor™ Violet 540 Dyes
an Excellent Replacement for Pacific Orange® and Krome Orange™ Dyes

Quick Summary	
Ex (nm)	399
Em (nm)	540
EC ($cm^{-1}M^{-1}$)	~21,000
$CF_{280\ nm}$*	0.661

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

mFluor™ Violet 540 dyes are an excellent replacement for Pacific Orange® and Krome Orange™ dyes since they have the spectral properties equivalent to those of Pacific Orange® and Krome Orange™. mFluor™ Violet 540 dyes are water-soluble, and some of the protein conjugates prepared with mFluor™ Violet 540 dyes are brighter than those prepared with Pacific Orange® and Krome Orange™. mFluor™ Violet 540 dyes and conjugates are excellent violet laser reagents for flow cytometry research. In addition, mFluor™ Violet SE is also an effective dye for labeling fixed or dead cells for flow cytometric application. The cells labeled with mFluor™ 540 can be viewed with the filter set of Pacific Orange™. mFluor™ Violet 540 may have less spillover effect than Pacific Orange® due to its slightly sharper emission.

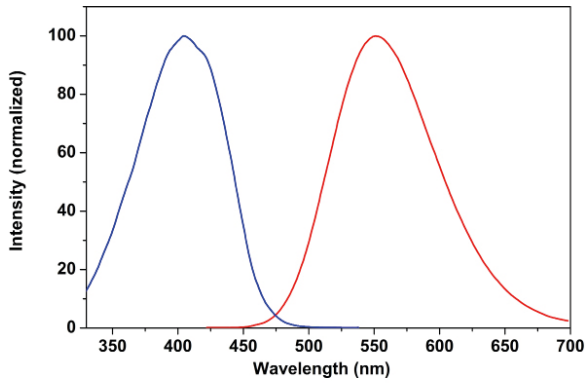

Figure 4.3 The excitation and emission spectra of mFluor™ Violet 540 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

mFluor™ Blue 570 Dyes
an Excellent Alternative to RPE

Quick Summary	
Ex (nm)	553
Em (nm)	570
EC ($cm^{-1}M^{-1}$)	170,000
$CF_{280\ nm}$*	0.191

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

mFluor™ Blue 570 dyes are an excellent alternative to RPE since they have the spectral properties equivalent to those of RPE conjugates. mFluor™ Blue 570 dyes are water-soluble, and the protein conjugates prepared with mFluor™ Blue 570 dyes are well excited at 488 nm to give red fluorescence (compatible with TRITC filter). mFluor™ Blue 570 dye and conjugates are excellent blue laser reagents for flow cytometry research. Although mFluor™ Blue 570 dyes are not as bright as RPE, they are much more photostable, making them useful for fluorescence imaging applications. It is very difficult to use RPE conjugates for fluorescence imaging applications due to the rapid photobleaching of RPE conjugates.

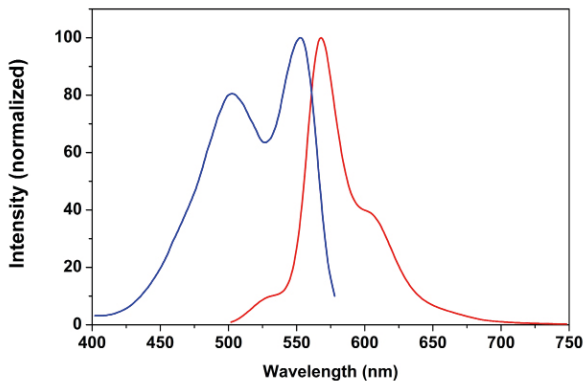

Figure 4.4 The excitation and emission spectra of mFluor™ Blue 570 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
20082	100 tests	Annexin V-mFluor™ Violet 540 conjugate	Pacific Orange® and Krome Orange™
1160	1 mg	mFluor™ Blue 570 SE	R-phycoerythrin (RPE)
16935	100 µg	mFluor™ Blue 570-streptavidin conjugate	R-phycoerythrin (RPE)
1152	1 mg	mFluor™ Violet 540 SE	Pacific Orange® and Krome Orange™
16932	100 µg	mFluor™ Violet 540-streptavidin conjugate	Pacific Orange® and Krome Orange™
1114	1 kit	ReadiLink™ mFluor™ Violet 540 protein labeling kit	Pacific Orange® and Krome Orange™

mFluor™ Green 620 Dye

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

Quick Summary	
Ex (nm)	522
Em (nm)	617
EC ($cm^{-1}M^{-1}$)	~60,000
$CF_{280\ nm}$*	0.554

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

mFluor™ Green 620 dye is a unique dye that is well excited by the green laser at 532 nm to give red fluorescence. mFluor™ Green 620 dye is water-soluble, and the protein conjugates prepared with mFluor™ Green 620 dye are bright with a Stokes shift of ~80 nm. mFluor™ Green 620 dye and conjugates are excellent green laser reagents for both flow cytometry research and fluorescence imaging applications. This small organic dye is much easier to be conjugated to a protein (such as monoclonal antibodies) than the equivalent fluorescent proteins (such as RPE, APC and PerCP) with high conjugation yield. This mFluor™ dye has been used to prepare CD antibody conjugates for multicolor flow cytometric cell analysis.

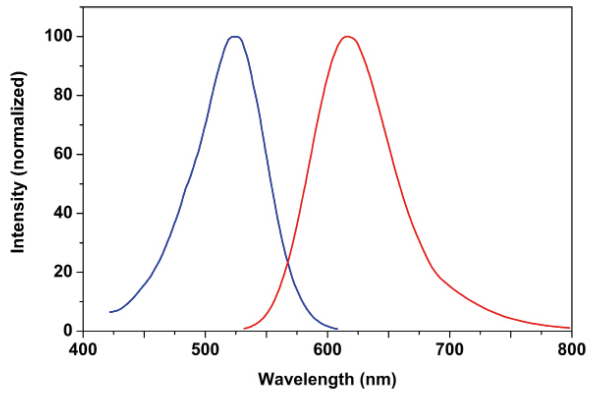

Figure 4.5 The excitation and emission spectra of mFluor™ Green 620 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

mFluor™ Yellow 630 Dye

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

Quick Summary	
Ex (nm)	561 (611)
Em (nm)	630
EC ($cm^{-1}M^{-1}$)	~110,000
$CF_{280\ nm}$*	0.391

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

mFluor™ Yellow 630 dye is a unique dye that is well excited by the green-yellow laser at 561 nm to give red fluorescence. mFluor™ Yellow 630 dye is water-soluble, and the protein conjugates prepared with mFluor™ Yellow 630 dye are bright. mFluor™ Yellow 630 dye and conjugates are excellent yellow laser reagents for both flow cytometry research and fluorescence imaging applications. This small organic dye can be readily conjugated to a variety of biological targets. We have used this dye conjugates for monitoring cell apoptosis and cell proliferation analysis. AAT Bioquest offers a variety of custom conjugation services with this unique dye.

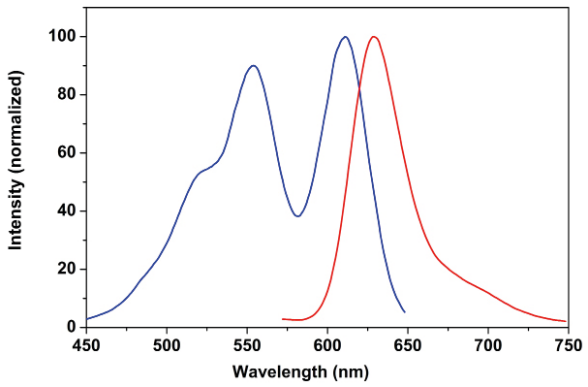

Figure 4.6 The excitation and emission spectra of mFluor™ Yellow 630 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name
1165	1 mg	mFluor™ Green 620 SE
16938	100 µg	mFluor™ Green 620-streptavidin conjugate
1170	1 mg	mFluor™ Yellow 630 SE
16942	100 µg	mFluor™ Yellow 630-streptavidin conjugate

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mFluor™ Red 700 Dyes
an Excellent Alternative to APC-Alexa Fluor® 700 Tandems

Quick Summary	
Ex (nm)	657
Em (nm)	700
EC ($cm^{-1}M^{-1}$)	295,000
$CF_{280\ nm}$*	0.096

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

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

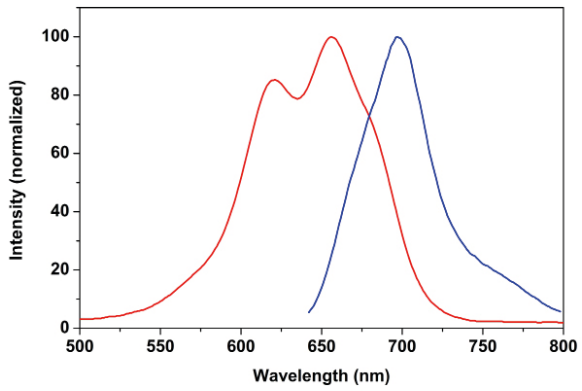

Figure 4.7 The excitation and emission spectra of mFluor™ Red 700 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

mFluor™ Red 780 Dyes
an Excellent Alternative to APC-Alexa Fluor® 750 Tandems

Quick Summary	
Ex (nm)	629
Em (nm)	780
EC ($cm^{-1}M^{-1}$)	~90,000
$CF_{280\ nm}$*	0.081

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins)

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

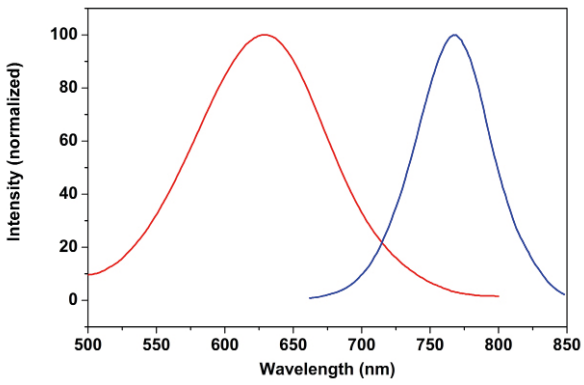

Figure 4.8 The excitation and emission spectra of mFluor™ Red 780 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name
1190	1 mg	mFluor™ Red 700 SE
16946	100 µg	mFluor™ Red 700-streptavidin conjugate
1191	1 mg	mFluor™ Red 780 SE
16948	100 µg	mFluor™ Red 780-streptavidin conjugate

AAT Bioquest Fluorochrome Reference Chart for Flow Cytom-

Excitation Laser / Emission Color	UV (355 nm)	Violet (405 nm)	Blue (488 nm)	Green (532 nm)	Yellow (561 nm)	Red (633 nm)
Blue	AMCA DAPI Hoechst Dyes iFluor™ 350	mFluor™ Violet 450 Nuclear Blue™ V450				
Green (FL1)		mFluor™ Violet 510	iFluor™ 488 FITC Nuclear Green™			
Yellow (FL2)		mFluor™ Violet 540	RPE Propidium Iodide (PI)	iFluor™ 514 Nuclear Orange™		
Orange			RPE-California Red™	iFluor™ 532		
Red (FL3)			7-AAD RPE-iFluor™ 647 PerCP	mFluor™ Green 620	mFluor™ Yellow 630	iFluor™ 647 APC Nuclear Red™
Far Red (FL4)			RPE-iFluor™ 700			mFluor™ Red 700 APC-iFluor™ 700
Infrared			RPE-iFluor™ 750			APC-iFluor™ 750 mFluor™ Red 780

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Phycobiliproteins and Their Tandem Conjugates 5

Phycobiliprotein Fluorochromes at-a-glance*

	APC	RPE	PerCP
Unconjugated	**2552** **2554**	**2558**	**2559**
iFluor™ 647 Tandem (Alternative to Cy5®, AF 647, DL 647)		**2577**	
iFluor™ 700 Tandem (Alternative to AF 700)	**2570**		
iFluor™ 750 Tandem (Alternative to Cy7®, AF 750, and DL 750)	**2571**	**2578**	
Streptavidin Conjugate	**16902** **16908**	**16900** **16901** **16906** **16907**	**16905**

* products listed by catalog number; AF = Alexa Fluor®; DL = DyLight™

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Phycobiliproteins and Their Tandem Conjugates

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 at 4 °C as ammonium sulfate precipitates. Purified biliproteins may dissociate into subunits under acidic or basic conditions, but are relatively stable at room temperature at neutral pH with concentrations greater than 0.1 mg/mL. Dissociated subunits typically have less intense coloration and fluorescence than the native pigment. It is recommended that all phycobiliproteins and their conjugates (preferably in neutral buffer solution) be refrigerated, never frozen.

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

B-Phycoerythrin (B-PE) has three absorption bands with maximum absorption at 545 nm. The subunit structure of B-PE is similar to that of R-PE, but the chromophore content of the subunits differs, causing the difference in the relative intensities of the absorption peaks: α and β subunits contain only phycoerythrobilins (PEB) while γ subunit contains PEB and phycourobilin (PUB). B-PE is found both in cyanobacteria and red algae. The intense pink color and orange fluorescence of B-PE are virtually indistinguishable from those of R-PE by naked eyes.

Allophycocyanin (APC) is the least stable among the major phycobiliproteins, susceptible to dissociation at low concentrations including concentrations at which some assays are performed. For this reason, many researchers prefer to use CL-APC which is chemically cross-linked between α and β subunits and much more stable than APC.

R-Phycoerythrin (R-PE) is isolated from red algae. Its primary absorption peak is at 565 nm with secondary peaks at 496 nm and 545 nm. The relative prominence of the secondary peaks varies significantly among R-PEs from different species. R-PE has three types of subunits: α (~20,000 daltons), β (~20,000 daltons) and γ (~30,000 daltons). The molecular weight of intact R-PE has been found to be about 240,000 daltons, and a subunit structure of $(\alpha\beta)_6\gamma$ has been determined. The α subunit of R-PE contains only the PEB chromophore, while β and γ subunits contain both PEB and PUB. Variability in the absorption spectra of R-PEs from various species reflects differences in the PEB/PUB ratio of the subunits. R-PE and closely related B-PE are the most intensely fluorescent phycobiliproteins, with quantum efficiencies probably in excess of 90%, and its orange fluorescence is readily visible by eye in any moderately concentrated solution.

C-Phycocyanin (C-PC) occurs as the major phycobiliprotein in many cyanobacteria and as a secondary phycobiliprotein in some red algae. The pigment has a single visible absorption maximum between 615 and 620 nm and a fluorescence emission maximum at ~650 nm. Its molecular weight is between 70,000 and 110,000 daltons. The pigment is composed of two subunits, α and β, which occur in equal numbers, but the exact number of α and β pairs which make up the molecule may vary among the species. Both α and β subunits contain only the PCB chromophore. In addition to absorbing light directly, this intensely blue pigment accepts quanta from phycoerythrin by fluorescent energy transfer in organisms in which PE is present. The red fluorescence of C-PC is transferred to allophycocyanin.

APC (Allophycocyanin) and Its Tandems

Description	Ex (nm)	Em (nm)
APC	651	662
APC-iFluor™ 700 tandem	651	713
APC-iFluor™ 750 tandem	651	780
CL-APC	651	662

APC is an accessory photosynthetic pigment found in blue-green algae. Its molecular weight is approximately 105 kDa. APC has six phycocyanobilin chromophores per molecule, which makes it a very bright fluorochrome that is highly suitable for flow cytometry applications. Our APC-iFluor™ 700 tandem is an excellent replacement for APC-Alexa Fluor® 700 tandem since they have almost identical spectra. On some antibodies, APC-iFluor™ 700 tandem is much brighter than APC-Alexa Fluor® 700 tandem with a higher stain index. Our APC-iFluor™ 750 tandem is an excellent replacement for APC-Cy7® and APC-Alexa Fluor® 750 tandem due to their similar spectral properties. APC-iFluor™ 750 tandem is more photostable than APC-Cy7® tandem.

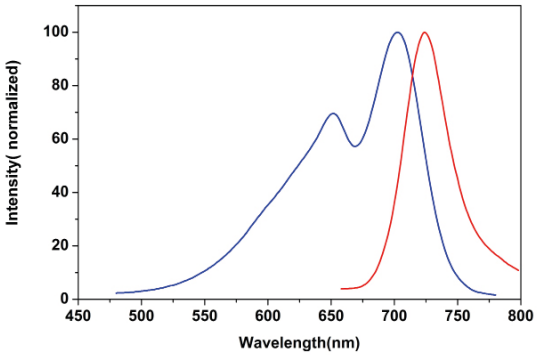

Figure 5.2 The absorbance and emission spectra of APC-iFluor™ 700 tandem (Cat# 2570) in PBS buffer (pH 7.2).

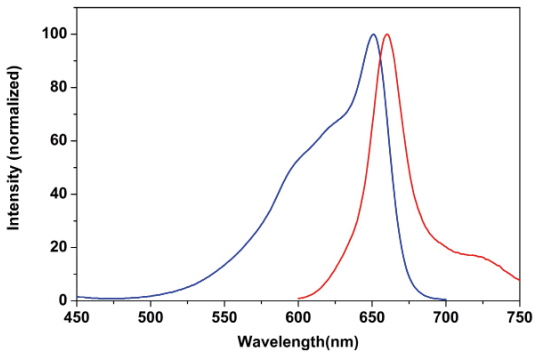

Figure 5.1 The absorbance and emission spectra of APC (Cat# 2554) in PBS buffer (pH 7.2).

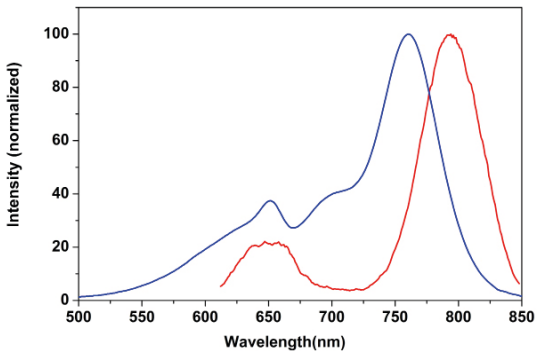

Figure 5.3 The absorbance and emission spectra of APC-iFluor™ 750 tandem (Cat# 2571) in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
2554	1 mg	APC (Allophycocyanin)	
2570	1 mg	APC-iFluor™ 700 tandem	APC-Alexa Fluor® 700 tandem
16908	100 µg	APC-iFluor™ 750-streptavidin conjugate	APC-Alexa Fluor® 750-streptavidin conjugate
2571	1 mg	APC-iFluor™ 750 tandem	APC-Alexa Fluor® 750 tandem
16902	100 µg	APC-streptavidin conjugate	
2552	1 mg	CL-APC (Cross linked-AlloPhycocyanin)	

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RPE (R-Phycoerythrin) and Its Tandems

Description	Ex (nm)	Em (nm)
RPE	496	575
RPE-California Red™ tandem	496	610
RPE-iFluor™ 647 tandem	496	674
RPE-iFluor™ 750 tandem	496	780

RPE is isolated from red algae. It has three types of subunits: α (~20,000 daltons), β (~20,000 daltons) and γ (~30,000 daltons). The molecular weight of intact RPE has been found to be about 240,000 daltons. RPE is the most intensely fluorescent phycobiliproteins with quantum efficiencies probably in excess of 90%. Its orange fluorescence is readily visible by eye in any moderately concentrated solution. Each RPE exists in vitro as a 240-kDa protein with 23 phycoerythrobilin chromophores per molecule. This makes RPE the brightest fluorochrome for flow cytometry applications. However, its photobleaching property makes RPE unsuitable for fluorescence microscopy.

Our RPE-iFluor™ 647 tandem is an excellent replacement for RPE-Cy5® and RPE-Alexa Fluor® 647 tandem since they have almost identical spectra while our RPE-iFluor™ 750 tandem is an excellent replacement for RPE-Cy7® and RPE-Alexa Fluor® 750 tandem due to their similar spectral properties. In general, our RPE tandems have less residual RPE fluorescence, and are more photostable (in some cases) than RPE-Cy7® and RPE-Alexa Fluor® 750 tandems. Our California Red™-RPE conjugate has flow cytometry performance superior to the commonly used RPE-Texas Red® tandem.

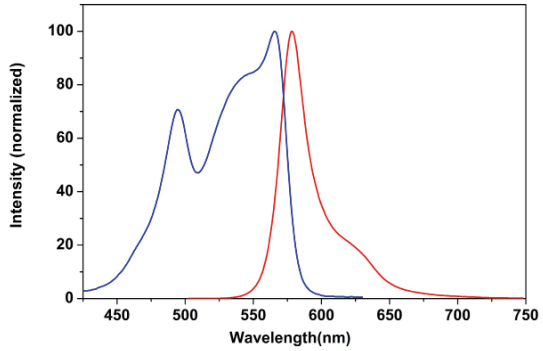

Figure 5.4 The absorbance and emission spectra of RPE (Cat# 2558) in PBS buffer (pH 7.2).

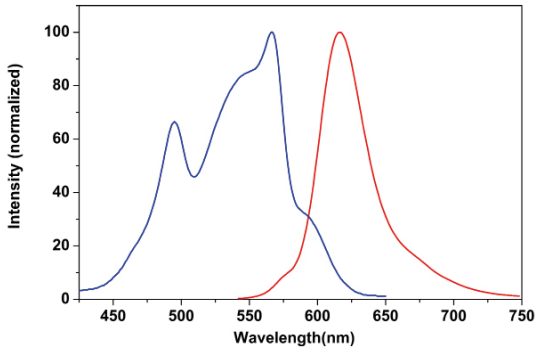

Figure 5.5 The absorbance and emission spectra of RPE-California Red™ tandem in PBS buffer (pH 7.2) .

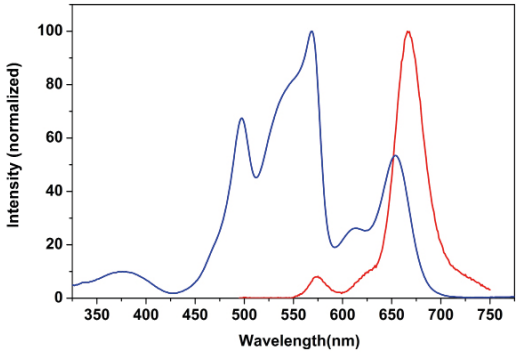

Figure 5.6 The absorbance and emission spectra of RPE-iFluor™ 647 tandem (Cat# 2577) in PBS buffer (pH 7.2).

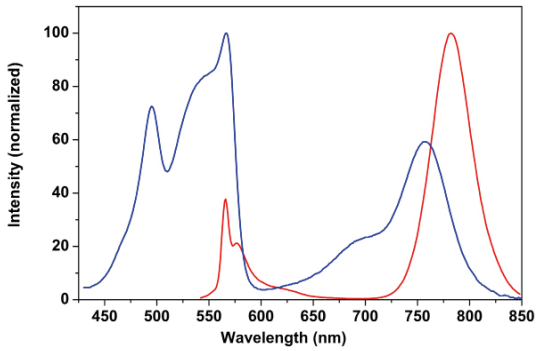

Figure 5.7 The absorbance and emission spectra of RPE-iFluor™ 750 tandem (Cat# 2578) in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name	Alternative to
2558	1 mg	RPE (R-Phycoerythrin)	
16906	100 µg	RPE-iFluor™ 647-streptavidin conjugate	RPE-Alexa Fluor® 647 tandem-streptavidin conjugate
2577	1 mg	RPE-iFluor™ 647 tandem	RPE-Alexa Fluor® 647 tandem
16907	100 µg	RPE-iFluor™ 750-streptavidin conjugate	RPE-Alexa Fluor® 750 tandem-streptavidin conjugate
2578	1 mg	RPE-iFluor™ 750 tandem	RPE-Alexa Fluor® 750 tandem
16900	100 µg	RPE-streptavidin conjugate	
16901	1 mg	RPE-streptavidin conjugate	

PerCP (Peridinin-Cholorophyll-Protein Complex) and Its Tandem

Description	Ex (nm)	Em (nm)
PerCP	482	677
PerCP-iFluor™ 700 tandem	482	713

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

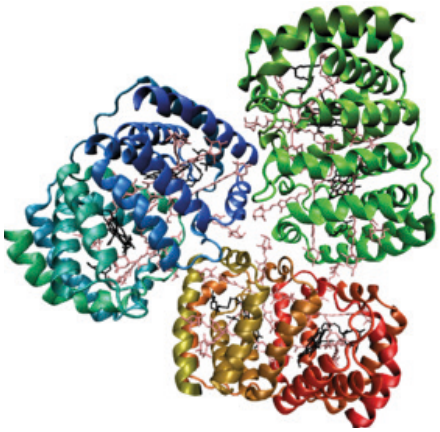

Figure 5.8 PerCP Crystal Structure

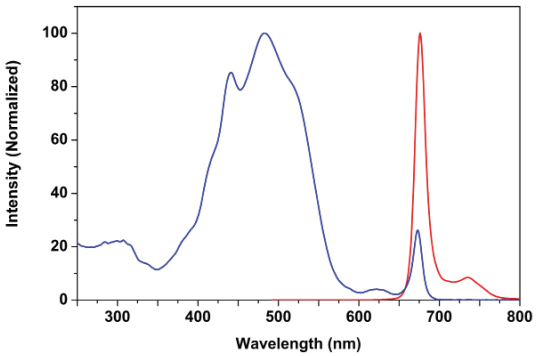

Figure 5.9 The absorbance and emission spectra of PerCP (Cat# 2559) in PBS buffer (pH 7.2).

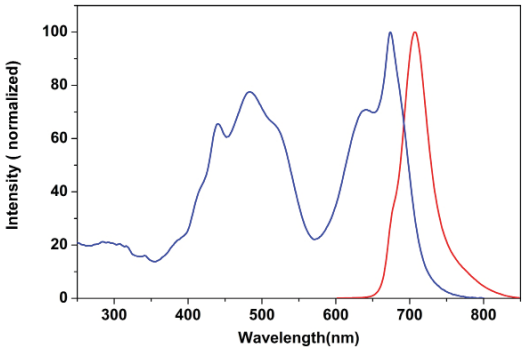

Figure 5.10 The excitation and emission spectra of PerCP-iFluor™ 700 Goat Anti-Rabbit IgG conjugate in PBS buffer (pH 7.2).

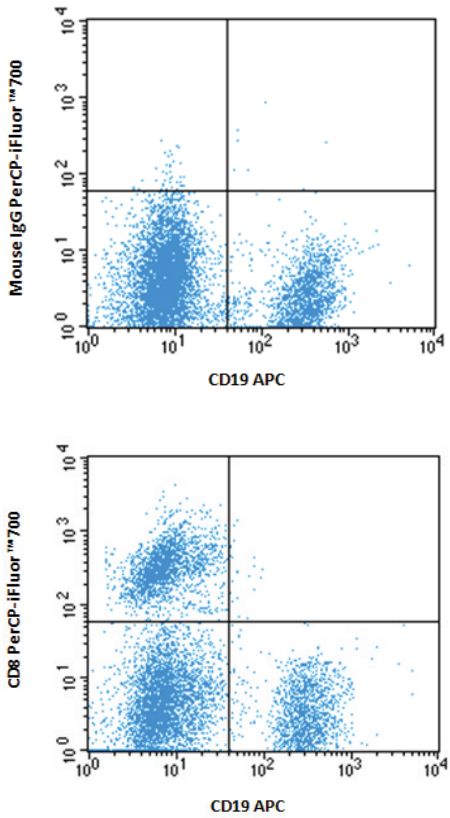

Figure 5.11 Staining of normal human peripheral blood cells with Anti-Human CD19 APC and Mouse IgG PerCP-iFluor™ 700 Control or Anti-Human CD8 PerCP-iFluor™ 700. Cells in the lymphocyte gate were used for analysis.

Product Ordering Information

Cat #	Size	Product Name
2559	1 mg	PerCP (Peridinin-Chlorophyll-Protein Complex)
2581	1 mg	PerCP- iFluor™ 700 tandem
16905	100 µg	PerCP-streptavidin conjugate

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trFluor™ Fluorescent Labeling Dyes

6

trFluor™ Labeling Dyes at-a-glance*

Donor	Acceptor
trFluor™ Eu	APC
• 1433 (NH_2-Reactive)	iFluor™ 647
• 1434 (SH-Reactive)	Tide Fluor™ 5 (TF 5)
• 1300 (Labeling Kit)	Alexa Fluor®647
trFluor™ Tb	iFluor™ 488
• 1443 (NH_2-Reactive)	Tide Fluor™ 2 (TF 2)
• 1444 (SH-Reactive)	Alexa Fluor®488
• 1305 (Labeling Kit)	FITC

* products listed by catalog number

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trFluor™ Fluorescent Labeing Dyes

Optimized for TR-FRET Applications

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 AAT Bioquest trFluor™ probes enable time-resolved fluorometry (TRF) for the assays that require high sensitivity. These trFluor™ probes have large Stokes shifts and extremely long emission half-lives when compared to traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared to other TRF compounds, our trFluor™ probes have relatively high stability, high emission yield and ability to be linked to biomolecules. Moreover, our trFluor™ Eu probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies.

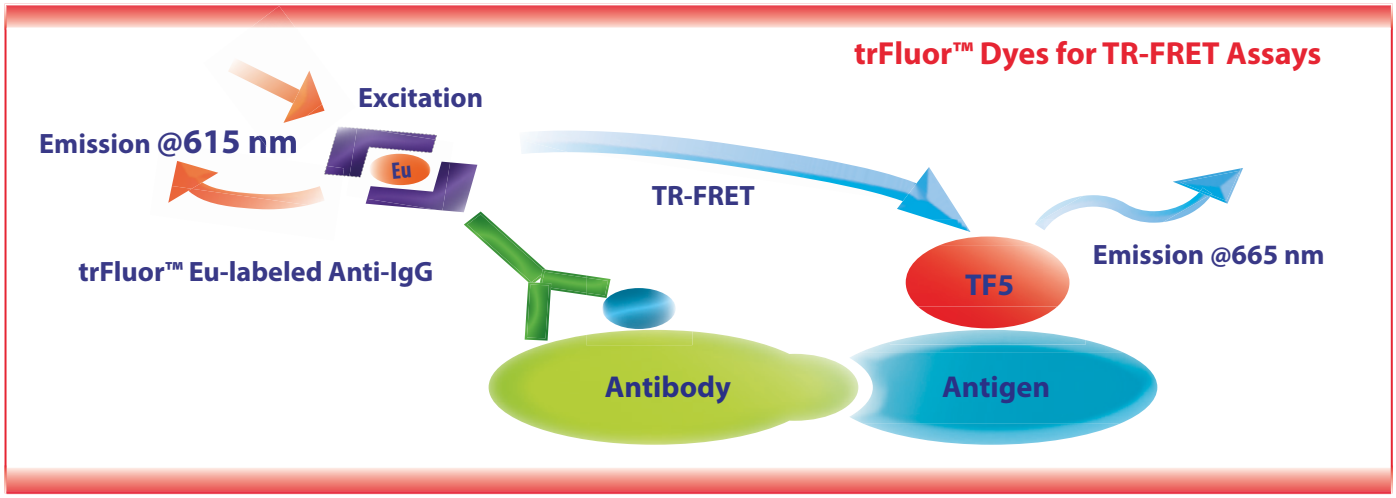
Key Features of trFluor™ Dyes:

- 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.

Table 6.1 Typical acceptors for the time-resolved luminescent probes

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

Figure 6.1 TR-FRET Assay Principle using trFluor™ Eu as the donor while Tide Fluor™ 5 (TF5) as the acceptor



trFluor™ Eu Dye
an Excellent Building Block for Developing TR-FRET Assays

Quick Summary	
Ex (nm)	346
Em (nm)	617
EC ($cm^{-1}M^{-1}$)	~22,000
$CF_{280\ nm}$*	0.777
$CF_{260\ nm}$**	0.911

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins);
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

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

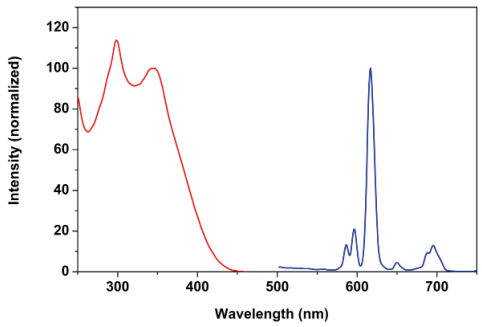


Figure 6.2 The excitation and emission spectra of trFluor™ Eu Goat Anti-Rabbit IgG conjugate (Cat# 16668) in PBS buffer (pH 7.2).

trFluor™ Tb Dye
an Excellent Building Block for Developing TR-FRET Assays

Quick Summary	
Ex (nm)	330
Em (nm)	544
EC ($cm^{-1}M^{-1}$)	~20,000
$CF_{280\ nm}$*	0.797
$CF_{260\ nm}$**	0.942

* $CF_{280\ nm} = EC_{280\ nm}/EC_{dye\ max}$ (Correction factor for peptides and proteins);
** $CF_{260\ nm} = EC_{260\ nm}/EC_{dye\ max}$ (Correction factor for oligos and nucleic acids)

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

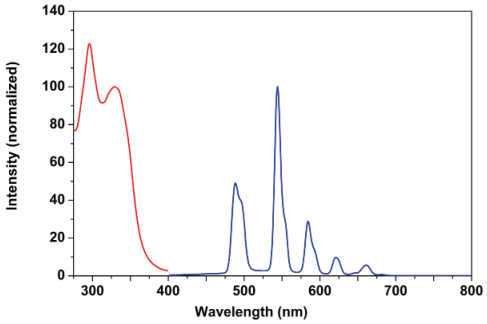


Figure 6.3 The excitation and emission spectra of trFluor™ Tb Goat Anti-Rabbit IgG conjugate (Cat# 16669) in PBS buffer (pH 7.2).

Product Ordering Information

Cat #	Size	Product Name
1300	1 kit	ReadiLink™ trFluor™ Eu protein labeling kit
1305	1 kit	ReadiLink™ trFluor™ Tb protein labeling kit
16518	100 µg	trFluor™ Eu goat anti-mouse IgG (H+L)
16668	100 µg	trFluor™ Eu goat anti-rabbit IgG (H+L)
1434	100 µg	trFluor™ Eu maleimide
16925	100 µg	trFluor™ Eu-streptavidin conjugate
1433	1 mg	trFluor™ Eu succinimidyl ester
16519	100 µg	trFluor™ Tb goat anti-mouse IgG (H+L)
16669	100 µg	trFluor™ Tb goat anti-rabbit IgG (H+L)
1444	100 µg	trFluor™ Tb maleimide
16926	100 µg	trFluor™ Tb-streptavidin conjugate
1443	1 mg	trFluor™ Tb succinimidyl ester

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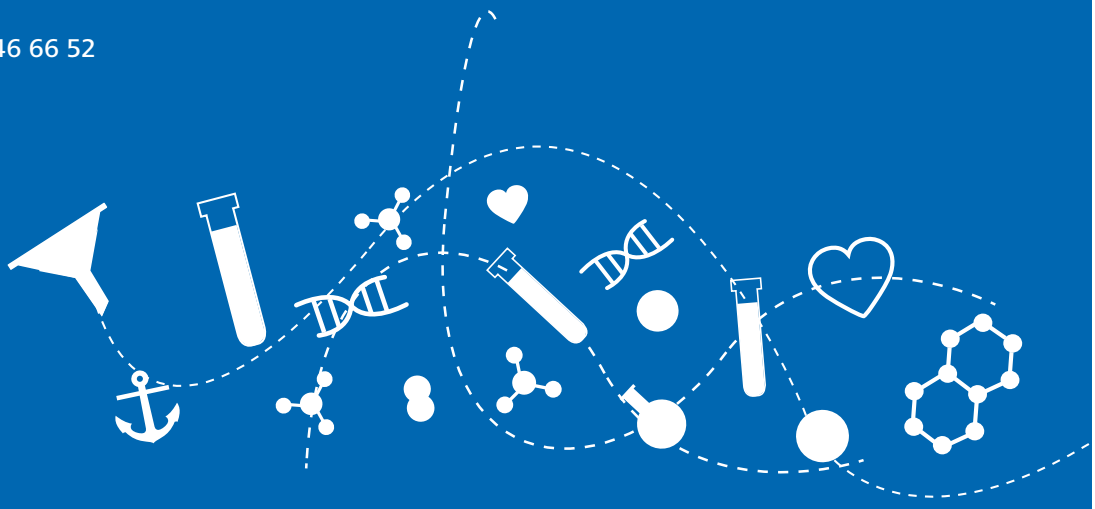
info@biomol.de · www.biomol.de

BIOMOL GmbH
Waidmannstraße 35 · 22769 Hamburg · Germany
info@biomol.de · www.biomol.de · www.biomol.com
Tel. 040-853 260 0 · Fax 040-853 260 22
Gebührenfrei:
Tel. 0800-246 66 51 · Fax 0800-246 66 52



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