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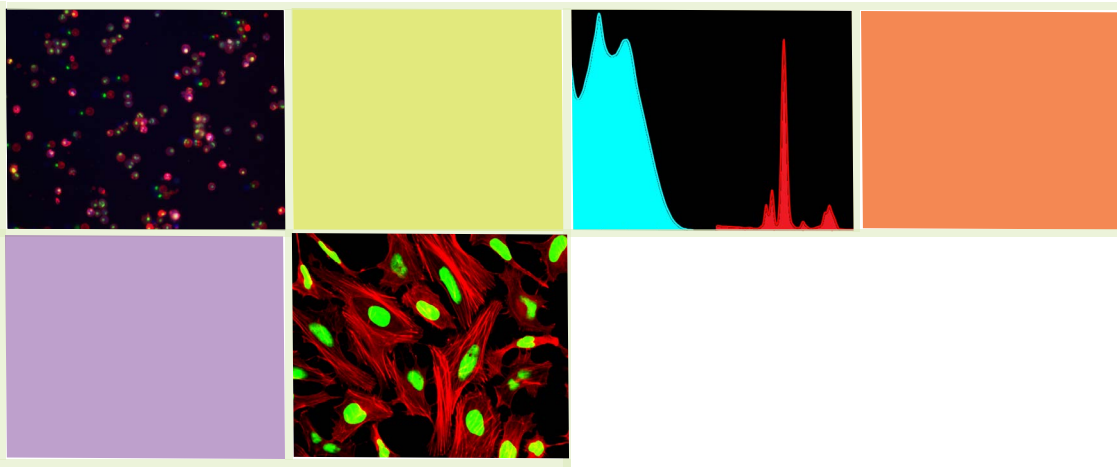
AssayWise Letters



AAT Bioquest®
Advancing Assay & Test Technologies

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From the President of AAT Bioquest

AAT Bioquest, Inc. (formerly ABD Bioquest, Inc.) develops, manufactures and markets bioanalytical research reagents and kits to life sciences, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. AAT Bioquest offers a rapidly expanding list of enabling products. ***AssayWise Letters*** is a platform for AAT Bioquest to introduce its newest products and services, and to update the new applications of our existing products. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest also offers custom service to meet the distinct needs of each customer.

It is my greatest pleasure to welcome you to this new issue of our ***AssayWise Letters***. While we continue to rapidly expand, our core value remains the same: **Innovation and Customer Satisfaction**. We are committed to being the provider of novel biological detection solutions. We promise you to extend these values to you during the course of our service and to continue to support you with our new products and services. It is our greatest honor to receive valuable feedback and suggestions from you.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

Monitoring Cell Cycles

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

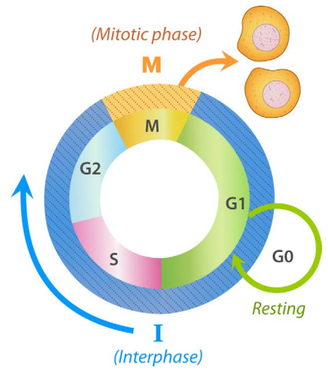

Figure 1.1. Cell division is just one of several stages that a cell goes through during its lifetime. The cell cycle is a repeating series of events that include growth, DNA synthesis, and cell division. The cell cycle in prokaryotes is quite simple: the cell grows, its DNA replicates, and the cell divides. In eukaryotes, the cell cycle is more complicated. The diagram above represents the cell cycle of a eukaryotic cell. As you can see, the eukaryotic cell cycle has several phases. The mitosis phase (M) actually includes both mitosis and cytokinesis. This is when the nucleus and then the cytoplasm divide. The other three phases (G1, S, and G2) are generally grouped together as an interphase. During the interphase, the cell grows, performs routine life processes, and prepares to divide.

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

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

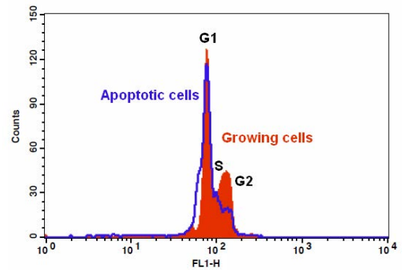

Figure 1.2. DNA profile in growing and camptothecin treated Jurkat cells. Jurkat cells were treated without (red) or with 20 μM camptothecin (blue) in a 37 °C, 5% CO_2 incubator for about 8 hours, and assayed with Cell Meter™ Fluorimetric Cell Cycle Assay kit (Cat# 22841). The fluorescence intensity of Nuclear Green™ LCS1 was measured with a FACSCalibur™ flow cytometer using the FL1 channel. In growing Jurkat cells, nuclei stained with Nuclear Green™ LCS1 showed G1, S, and G2 phases (red). In camptothecin treated apoptotic cells (blue), the fluorescence intensity of Nuclear Green™ LCS1 was decreased, and both S and G2 phases were diminished.

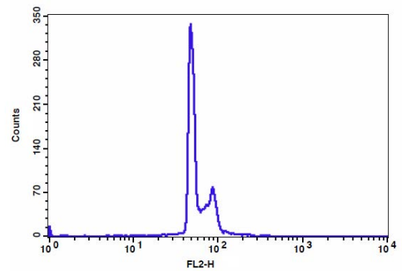

Figure 1.3. DNA profile in growing Jurkat cells. Jurkat cells were dye-loaded with Cell Meter™ Fluorimetric Cell Cycle Assay kit (Cat# 22842) and RNase A for 30 minutes. The fluorescence intensity of Nuclear Red™ LCS1 was measured with the FACSCalibur™ (Becton Dickinson, San Jose, CA) flow cytometer using the FL2 channel.

Table 1.1. Cell Cycle Assay Kits

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
22841	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	503	526
22842	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 tests	535	617

Multicolor Labeling of Cell Nucleus with DNA-Binding Dyes

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

Labeling the Nuclei of Live Cells

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

Our recently developed Nuclear Blue™ LCS1 is a fluorogenic, DNA-selective and cell-permeant dye for analyzing DNA content in living cells. The Nuclear Blue™ LCS1 has its blue fluorescence significantly enhanced upon binding to DNA. It can be used in fluorescence imaging, microplate and flow cytometry applications. It is well excited by violet laser at 405 nm, and emits blue/cyan fluorescence light with an emission maximum at ~440 nm, and provides an excellent tool for flow cytometers equiped with a 405 nm violet laser source. This DNA-binding dye might be used for multicolor analysis of live cells with the filter sets of Pacific Blue™ and BD Horizon™ V450.

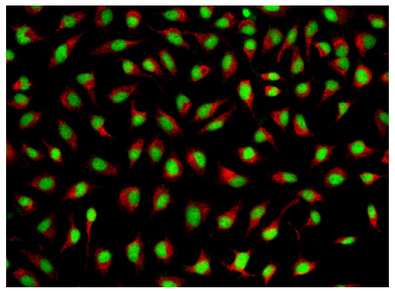

Figure 1.4. Image of live HeLa cells stained with Nuclear Green™ LCS1 (Cat# 17540). The mitochondria of live HeLa cells were stained with red fluorescence Cell Navigator™ Mitochondrion Staining Kit (Cat# 22668).

Labeling the Nuclei of Dead Cells

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

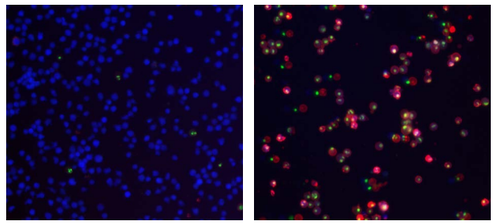

Figure 1.5. Binding activity of Apopxin™ Deep Red to phosphatidylserine in Jurkat cells. The fluorescence images demonstrated that live cells (blue) were stained by CytoCalcein™ Violet 450 (Cat# 22012), apoptotic cells (red) were stained by Apopxin™ Deep Red, and necrotic cells (green) were stained by Nuclear Green™ DCS1 (Cat# 17550). Apoptosis was induced by 1 µM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope using the violet, Cy5® and FITC channel respectively. A: Non-induced control cells; B: Triple staining of staurosporine-induced cells.

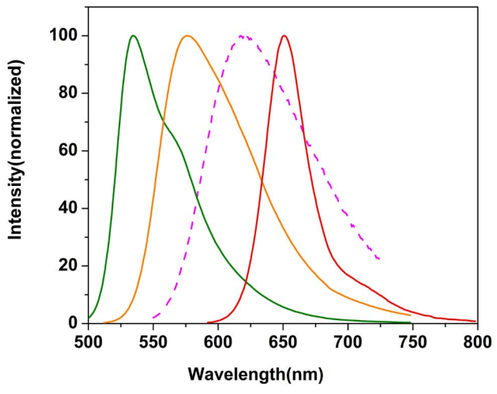

Figure 1.6. The normalized emission spectral comparison of Nuclear Green™ DCS1 (Ex/Em = 503/526 nm, Cat# 17550), Nuclear Orange™ DCS1 (Ex/Em = 514/555 nm, Cat# 17551), and Nuclear Red™ DCS1 (Ex/Em = 622/645 nm, Cat# 17552) in the presence of calf thymus DNA. The dotted line is emission spectrum of propidium iodide bound to DNA (Ex/Em = 535/617 nm, Cat# 17515).

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New Product Highlights

Table 1.2 Cell Nuclear Stains

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
17501	7-AAD [7-Aminoactinomycin D]	1 mg	546	647
17510	DAPI [4,6-Diamidino-2-phenylindole, dihydrochloride] *UltraPure Grade*	10 mg	358	461
17562	Hexidium Iodide	5 mg	518	600
17520	Hoechst 33258 *UltraPure Grade*	100 mg	352	461
17530	Hoechst 33342 *UltraPure Grade*	100 mg	350	461
17537	Hoechst 34580 *UltraPure Grade*	5 mg	368	437
17514	Hydroxystilbamidine	10 mg	360	625
17561	LDS 751	25 mg	543	712
17543	Nuclear Blue™ LCS1	0.5 mL	401	459
17550	Nuclear Green™ DCS1	0.5 mL	503	526
17540	Nuclear Green™ LCS1	0.5 mL	503	526
17551	Nuclear Orange™ DCS1	0.5 mL	528	576
17541	Nuclear Orange™ LCS1	0.5 mL	514	555
17552	Nuclear Red™ DCS1	0.5 mL	631	651
17542	Nuclear Red™ LCS1	0.5 mL	622	645
17539	Nuclear Yellow [Hoechst S769121]	25 mg	355	495
17515	Propidium Iodide *UltraPure Grade*	25 mg	535	617

DAX-J2™ Ratio 580/460, a Ratiometric Nitric Oxide (NO) Probe

DAX-J2™ Ratio 580/460 is a new nitric oxide (NO) sensor recently developed by AAT Bioquest. It is a cell permeable reagent that can measure free NO and nitric oxide synthase (NOS) activity in living cells in a ratiometric mode. Once inside the cell the blocking groups on the DAX-J2™ reagent are released to induce fluorescence ratio changes at wavelengths of 580 and 460 nm upon NO oxidation. The fluorescence intensities at 580 nm and 460 nm can be detected using the filter sets of Cy3®/TRITC and BD Horizon™ V450/ Pacific Blue. Most of flow cytometers and fluorescence microscopes are equipped with these two filter sets. DAX-J2™ Ratio 580/460 has distinct advantages for NO detection over the popular DAF-2 NO probe: 1). DAX-J2™ Ratio 580/460 does not require esterase activity for NO detection. DAF-2 requires intracellular esterases to cleave its acetate groups for detecting NO activity. 2). DAX-J2™ product exhibits pH-independent fluorescence while DAF-2 has its fluorescence highly affected by pH. 3). DAX-J2™ Ratio 580/460 can be monitored in a ratiometric mode.

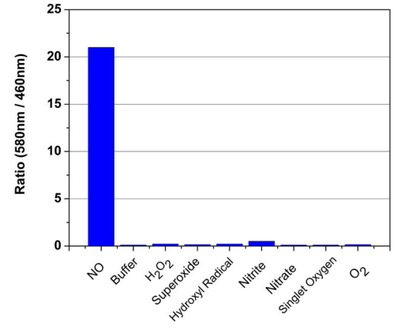

Figure 1.7. Fluorescence response of DAX-J2™ Ratio 580/460 (2 µM) to different reactive oxygen species (1 mM) in PBS buffer (pH = 7.2). The fluorescence intensities were measured at 580 nm and 460 nm respectively.

Table 1.3. Multicolor Nitric Oxide (NO) Detection Probes

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
16302	DAX-J2™ IR	1 mg	780	800
16300	DAX-J2™ Orange	1 mg	545	576
16310	DAX-J2™ Ratio 580/460	1 mg	420/540	460/580
16301	DAX-J2™ Red	1 mg	588	610

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Neuraminidase Assay

Neuraminidases, also called sialidases, are glycoside hydrolase enzymes that catalyze the hydrolysis of terminal sialic acid residues and neuraminic acids. The most commonly known neuraminidase is the viral neuraminidase. The cleavage of linkage between sialic acid and adjacent sugar residue permits the transport of the virus through mucin and destroys the haemagglutinin receptor on the host cell, thus allowing elution of progeny virus particles from infected cells. Neuraminidase promotes influenza virus release from infected cells and facilitates virus spread within the respiratory tract. Thus, it is an important target for influenza drug development. The detection of neuraminidase and screening its inhibitors is one of the essential tasks for investigating biological processes and prevention of influenza infection. Neuraminidase inhibitors are useful for combating influenza infection. Zanamivir (administered by inhalation), Oseltamivir (administered orally) and Peramivir (administered parenterally, through intravenous or intramuscular injection) are a few known neuraminidase inhibitors that are widely used for treating influenza infection.

There are a few assay kits available for detecting neuraminidase, but all the commercially available kits are tedious to use. Amplite™ Fluorimetric Neuraminidase Assay Kit provides a sensitive and robust fluorimetric assay to detect neuraminidase that exists either in cells or biological samples. The non-fluorescent neuraminidase substrate becomes strongly fluorescent upon neuraminidase cleavage. The kit can detect as little as 0.3 mU/mL neuraminidase in a 100 µL assay volume. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The signal can be easily read by a fluorescence microplate reader at Ex/Em = ~320/450 nm.

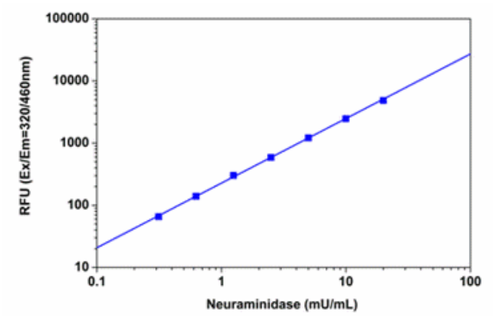

Figure 2.1. Neuraminidase dose responses were measured in a 96-well black plate with Amplite™ Fluorimetric Neuraminidase Assay Kit (Cat# 12602) using a Germini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU/mL of neuraminidase can be detected with 1 hour incubation in a 37 ºC, 5% CO_2 incubator.

Table 2.1 Neuraminidase Assay Kit

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
12602	Amplite™ Fluorimetric Neuraminidase Assay Kit *Blue Fluorescence*	200 tests	360	449

Sphingomyelinase Assay

Sphingomyelinase (SMase) is an enzyme that is responsible for cleaving sphingomyelin (SM) to phosphocholine and ceramide. Activation of SMases in cells plays an important role in the cellular responses. Five types of sphingomyelinase (SMase) have been identified based on their cation dependence and pH optima of action. Among the five types, the lysosomal acidic SMase and the magnesium-dependent neutral SMase are considered major candidates for the production of ceramide in the cellular response to stress.

Amplite™ Sphingomyelinase Assay Kits 13620 and 13621 provide sensitive methods for detecting neutral SMase activity or screening its inhibitors. They can be used for measuring the SMase activity in blood, cell extracts or other solutions. For Kit 13620, the absorbance of light at 595 nm is proportional to the formation of phosphocholine, therefore to the SMase activity. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments. For Kit 13621, it uses Amplite™ Red as a fluorogenic probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin (SM) by sphingomyelinase (SMase). The fluorescence intensity of Amplite™ Red is proportional to the formation of phosphocholine, therefore to the SMase activity. Amplite™ Red enables the assay readable either in fluorescence intensity or absorption mode.

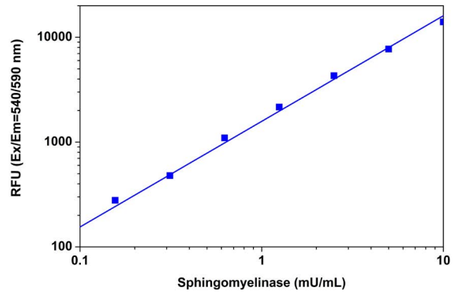

Figure 2.2. Sphingomyelinase dose responses were measured in a 96-well black solid plate with Amplite™ Fluorimetric Sphingomyelinase Assay Kit (Cat# 13621) using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.15 mU/mL of sphingomyelinase can be detected with 60 minutes incubation (n=3).

Table 2.2 Sphingomyelinase Assay Kits

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
13620	Amplite™ Colorimetric Sphingomyelinase Assay Kit *Blue Color*	200 tests	655	N/A
13622	Amplite™ Fluorimetric Acidic Sphingomyelinase Assay Kit *Red Fluorescence*	200 tests	571	585
13625	Amplite™ Fluorimetric Sphingomyelin Assay Kit *Red Fluorescence*	100 tests	571	585
13621	Amplite™ Fluorimetric Sphingomyelinase Assay Kit *Red Fluorescence*	200 tests	571	585

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Biochemical Assays

Multiplexing Caspase Activity Detection

A distinctive feature of the early stages of apoptosis is the activation of caspase enzymes. Members of the caspase (CED-3/ICE) family of cysteine–aspartic acid specific proteases have been identified as crucial mediators of the complex biochemical events associated with apoptosis. The recognition site for caspases is marked by three to four amino acids followed by an aspartic acid residue, with the cleavage occurring after the aspartate. The caspase proteases are typically synthesized as inactive precursors. Inhibitor release or cofactor binding activates the caspases through cleavage at internal aspartates, either by autocatalysis or by the action of another protease.

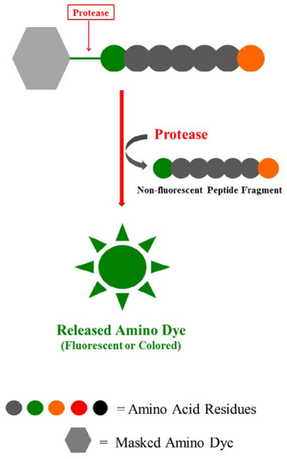

Figure 2.3. The caspase-sensitive peptide fragment-masked amino dyes are digested by a caspase to generate the highly fluorescent dye (or a highly colored dye). The fluorescence (or color) intensity increase is proportional to the caspase activity.

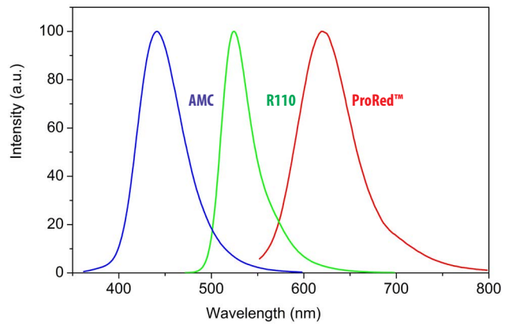

Figure 2.4. The normalized fluoresence spectra of AMC, R110 and ProRed™ in aqueous buffer (pH 7.0). AMC, R110 and ProRed™ caspase substrates are well suited for multiplexing caspase activities.

AAT Bioquest offers a diverse selection of capase inhibitors, chromogenic and fluorogenic caspase substrates, and caspase assay kits. Our chromogenic caspase substrates are based on 4-nitroaniline (4-PNA). AAT Bioquest is the only company that offers the multicolor substrates of four distinct fluorescence colors based on 7-Amino-4-methylcoumarin (AMC), 7-Amino-4-trifluoromethylcoumarin (AFC), Rhodamine 110 (R110) and ProRed™ respectively. In particular, the ProRed™-based caspase substrates are extremely useful for screening caspase inhibitors due to their longer excitation and emission wavelengths that eliminate the autofluorescence interference from the compound library.

AAT Bioquest has developed Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit (Cat# 22820) for multiplexing the detection of caspases 3, 7, 8 and 9. This particular kit is designed to simultaneously monitor four key caspases (caspase 3, 7, 8 and 9) activation involved in cell apoptosis using the three distinct fluorescent colors. This kit uses DEVD-ProRed™, IETD-R110 and LEHD-AMC as fluorogenic indicators for caspase 3/7, 8 and 9 activity respectively. Upon caspase cleavages, DEVD-ProRed™, IETD-R110 and LEHD-AMC caspase substrates generate three distinct fluorophores: ProRed™ (red fluorescence), R110 (green fluorescence) and AMC (blue fluorescence), which can be readily monitored in a single assay due to their nice spectral separation.

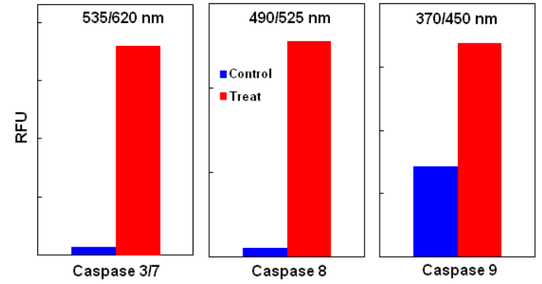

Figure 2.5. Detection of caspase activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with staurosporine at the final concentration of 1 µM for 4 hours (Red Bar) while the untreated cells were used as control (Blue Bar). The fluorescence intensity was measured with FlexStation fluorescence microplate reader at the indicated wavelength. The caspase 3/7, 8 and 9 activities can be detected in a single assay without interferences from other caspases.

Table 2.3 Multiplexing Caspase Activity and Apoptosis Assay Kits

Cat. #	Product Description	Size
22820	Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit *Triple Fluorescence Colors*	3x100 tests
22850	Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit *Triple Fluorescence Colors*	100 tests

Ratio Imaging of Cells with RatioWorks™ Probes

Intracelluar Fluorescence Ratiometric Imaging

Many fundamental functions of a cell strongly depend on delicate, but nevertheless dynamic balances of ions (e.g. calcium, magnesium), voltage potentials and pH between the cell's cytosol and the surrounding extracellular space. Changes in these balances significantly alter a cell's behavior and function. Therefore, measurements of intracellular ion, voltage and pH dynamics in real time are of tremendous interest for researchers in neuroscience, cell biology and cell physiology in general. In many cases, however, exact estimations of actual ion concentrations or relative changes in different locations in a cell or a cellular network are difficult with conventional fluorescence methods. The reason is that these methods do not take account of the fact that differences in cell morphology within different parts of a single cell or between cell types in cellular networks might influence the quality and quantity of emitted light. This can lead to substantial misinterpretations when dynamic changes of ion concentrations, voltage or pH are investigated. Ratiometric imaging techniques bypasses these issues by observing emission or excitation wavelength shifts of fluorophores or by comparing the emission or excitation intensity of a fluorophore combination instead of measuring mere intensity changes.

Research activities are increasingly focusing on the identification and the spatial and temporal distribution of e.g. local "hot spots" for dynamic changes in ion concentration, voltage or pH in a cell or a cellular network. Such "hot spots" are often localized in specialized parts of a cell or in certain cells in a cellular network. Additionally, these areas often have different properties compared to the rest of the specimen in terms of cell metabolism or structure. Conventional fluorophores used to investigate dynamic physiological states change their emission intensity upon ion binding, pH change or voltage change (e.g. fluo-4 has increased emission upon calcium binding). However, these markers do not take into account that differences in structure, diameter or marker uptake/expression can cause changes in the quantity of emitted light that are not in correlation with the actual ion concentration, voltage or pH. To quantitatively and comparably detect the changes in cellular structures or different cells, a method insensitive to structure diameter and fluorophore concentration is needed. Ratiometric imaging offers the opportunity to reproducibly measure absolute intracellular ion, voltage and pH levels/changes with respect to cell diameter, fluorophore concentration and optical properties of the imaging setup. However, ratiometric imaging depends on a fast change of excitation wavelength or the detected wavelength, a strong light source, excellent transmission of optical components and fast signal detection. The recent development of ultrafast filter wheels, UV-light optimized objectives, highly sensitive fluorophores and new CCD cameras allows affordable quantitative high speed live cell imaging in high spatial resolution.

As mentioned above, in ratiometric imaging an emission shift instead of mere intensity change is imaged. To measure emission shifts, intensity changes of a fluorophore or fluorophore combination have to be measured either by using two different excitation wavelengths or by detecting at two different emission wavelengths. In the case of the commonly used calcium imaging dye Fura-2, the dye has to be excited with light at wavelengths of 340 nm and 380 nm and the detection wavelength is 510 nm. In contrast to that, the calcium imaging dye Indo-1 is usually excited with light at 350 nm wavelength and the detection wavelengths are 405 nm and 485 nm.

Fura-8™ for Ratiometric Calcium Detection

Although Fura-2 has been widely used as the preferred excitation-ratioable calcium indicator, it has certain limitations, e.g., lower sensitivity compared to the single wavelength calcium dyes such as Fluo-8® and Cal-520™. AAT Bioquest has recently developed Fura-8™ to improve the calcium response of Fura-2. As demonstrated in Figures 3.1 and 3.2, Fura-8™ AM is more sensitive to calcium than Fura-2 AM. In addition, Fura-8™ has its emission shifted to longer wavelength (Em = 525 nm). Fura-8™ might be also used for the flow cytometric analysis of calcium in cells due to its excellent excitation at 405 nm of violet laser.

Key Features of Fura-8™ Calcium Indicator

- *Fura-8™ responses to calcium the same way as Fura-2 does*
- *Red-shifted dual excitation wavelengths (354 nm/415 nm)*
- *Better excited at 405 nm for flow cytometric applications*
- *Compatible with common filter sets.*
- *Higher signal/background ratio than that of Fura-2*

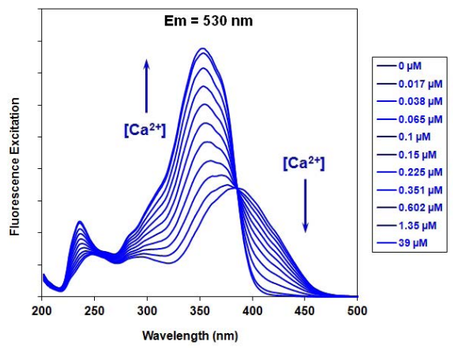

Figure 3.1. Fluorescence excitation spectra of Fura-8™ in solutions containing 0 to 39 µM free Ca^{2+}

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Cell-Based Assays

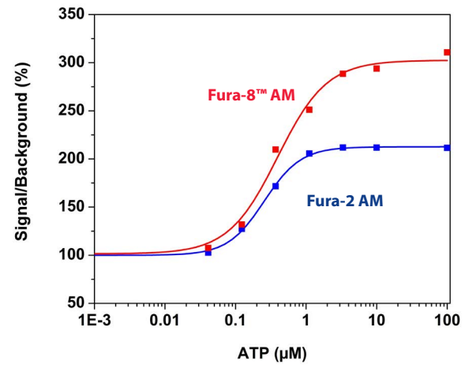

Figure 3.2. ATP dose responses in CHO-K1 cells measured with Fura-2 AM (Cat# 21025) and Fura-8™ AM (Cat# 21055) respectively. CHO-K1 cells were seeded overnight at 40,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were incubated with Fura-2 AM or Fura-8™ AM calcium assay dye-loading solution for 1 hour at room temperature. ATP (50 µL/well) was added by FlexStation®.

PDMPO, an Unique Dual Excitation and Dual Emission Ratiometric pH Indicator

The existing pH probes are unsuitable to study acidic organelles such as lysosomes, endosomes, spermatozoa and acrosomes because their fluorescence is significantly reduced at lower pH. In addition, most of the existing pH probes (such as BCECF and SNARF) are not selectively localized in acidic organelles. The growing potential of ratio imaging is significantly limited by the lack of appropriate fluorescent probes for acidic organelles although ratio imaging has received intensive attention in the past few decades. PDMPO [2-(4-pyridyl)-5-((4-(2-dimethylaminoethyl-aminocarbamoyl)methoxy)phenyl)oxazole] is characterized as an acidotropic dual-excitation and dual-emission pH probe. It emits intense yellow fluorescence at lower pH and gives intense blue fluorescence at higher pH. This unique pH-dependent fluorescence makes PDMPO an ideal pH probe for acidic organelles with pK_a = 4.47. PDMPO selectively labels acidic organelles (such as lysosomes) of live cells and the two distinct emission peaks can be used to monitor the pH fluctuations of live cells in ratio measurements. Additionally, the very large Stokes shift and excellent photostability of PDMPO make it an excellent fluorescent acidotropic reagent for fluorescence imaging. The unique fluorescence properties of PDMPO might give researchers a new tool with which to study the acidic organelles of live cells. PDMPO can be well excited by the violet laser at 405 nm for flow cytometric applications.

Although BCECF and BCFL dextrans are useful for detecting translocation into compartments that have an acidic pH; their relative insensitivity to fluorescence change below pH ~6 limits quantitative pH estimation. The lower pK_a values of the PDMPO dextran conjugate make it a more suitable indicator for estimating the pH of relatively acidic lysosomal environments. Moreover, the shift in its excitation and emission spectra in acidic media permits ratiometric pH measurements.

Our PDMPO dextran conjugates can be used to quickly and accurately estimate the pH of lysosomes. As the labeled dextran is

Table 3.1 Ratiometric Fluorescent Calcium Indicators

Cat #	Product Description	Size	Zero Calcium		High Calcium		K_d (nM)
			Ex (nm)	Em (nm)	Ex (nm)	Em (nm)	
21054	BTC AM	1 mg	464	533	401	529	7,000
21053	BTC, tetrapotassium salt	1 mg	464	533	401	529	7,000
21021	Fura-2 AM *UltraPure grade*	1 mg	363	512	335	505	145
21025	Fura-2, pentapotassium salt	1 mg	363	512	335	505	145
21026	Fura-2, pentasodium salt	1 mg	363	512	335	505	145
21055	Fura-8™ AM	1 mg	386	532	354	524	260
21056	Fura-8™ AM	10x50 µg	386	532	354	524	260
21057	Fura-8™, potassium salt	1 mg	386	532	354	524	260
21058	Fura-8™, sodium salt	1 mg	386	532	354	524	260
21032	Indo-1 AM *UltraPure grade*	1 mg	346	475	330	401	230
21040	Indo-1, pentapotassium salt	1 mg	346	475	330	401	230
21044	Indo-1, pentasodium salt	1 mg	346	475	330	401	230
21050	Quin-2 AM	1 mg	353	495	333	495	60
21052	Quin-2, tetrapotassium salt	5 mg	353	495	333	495	60

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taken up by the cells and moves through the endocytic pathway, the PDMPO fluorescence changes from blue in the near-neutral endosomes to yellow in the acidic lysosomes. The greatest change in fluorescence emission occurs near the pK_a of the dye at pH ~4.2. The pH in lysosomes can be measured with PDMPO dextrans using fluorescence microscopy or flow cytometry.

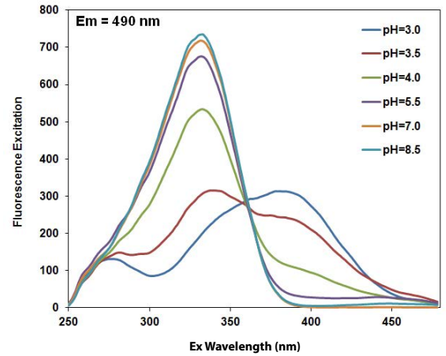

Figure 3.3. pH titration of PDMPO. Top: Excitation; Bottom: Emission.

Table 3.2 Reactive Fluorescent pH Probes and Their Dextran Conjugate

Cat #	Product Description	Size	Ex (nm)	Em (nm)
21217	Protonex™ Green 500 Dextran	1 mg	443	505
21216	Protonex™ Green 500, SE	1 mg	443	505
21209	Protonex™ Red 600-Latex Bead Conjugate	1 mL	575	597
21208	Protonex™ Red 600, SE	1 mg	575	597
21210	RatioWorks™ PDMPO, SE	1 mg	405	550
21211	RatioWorks™ PDMPO Dextran	1 mg	405	540

BCFL AM, a Superior Replacement to BCECF AM

BCFL AM is developed to overcome the isomer difficulty associated with BCECF AM. As BCECF AM, BCFL AM exhibits pH-dependent dual excitations, essentially identical to those of BCECF AM. It has a pK_a of ~7.0, identical to BCECF AM too. As with BCECF AM, the dual excitation spectrum of BCFL AM with an isosbestic point at 454 nm should make BCFL AM a good excitation-ratiometric pH indicator. BCFL ratiometric imaging makes intracellular pH determination essentially independent of several variable factors, including dye concentration, path length, cellular leakage and photobleaching rate. BCFL AM is a single isomer, making the pH measurement much more reproducible than BCECF AM, which is consisted of quite a few different isomers.

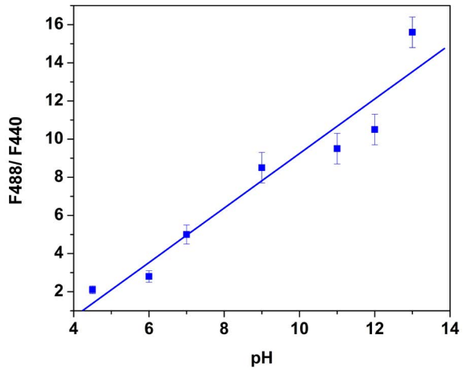

Figure 3.4. The fluorescence excitation ratios of BCFL at 488 nm and 440 nm were measured with 530 nm emission at pH 4.5, 6, 7, 9, 11, 12 and 13 using standard buffer solutions. The higher the pH, the higher the fluorescence signal with longer excitation wavelength (488 nm), and the lower the fluorescence signal with shorter excitation wavelength (440 nm).

Table 3.3 Fluorescent pH Probes for Near -Neutral pH Environments

Cat #	Product Description	Size	pK_a	Ex (nm)	Em (nm)
21201	BCECF Acid	1 mg	7.0	503	528
21202	BCECF AM	1 mg	7.0	503	528
21203	BCECF AM *UltraPure Grade*	20x50 µg	7.0	505	520
21180	Cell Meter™ Fluorimetric Intracellular pH Assay Kit	1000 tests	N/A	503	528
21189	RatioWorks™ BCFL Acid *Superior Replacement to BCECF*	1 mg	7.0	503	528
21190	RatioWorks™ BCFL AM *Superior Replacement to BCECF AM*	1 mg	7.0	503	528
21191	RatioWorks™ BCFL SE	1 mg	7.0	503	528

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Cell-Based Assays

Multicolor Labeling of Dead Cells for Flow Cytometric Analysis

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

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

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

Key Features of Fixable Dead Cell Staining Kits

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

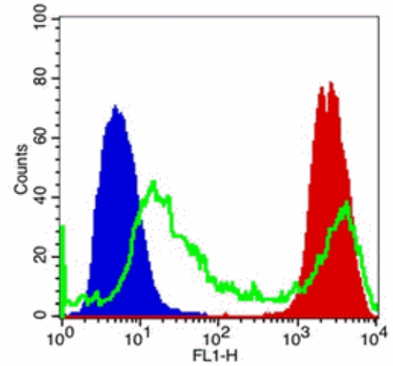

Figure 3.5. Detection of Jurkat cell viability using Cell Explorer™ Fixable Dead Cell Staining Kit (Cat# 22601). Jurkat cells were treated and stained with Stain IT™ Green and then fixed with 3.7% formaldehyde and analyzed by flow cytometry. Live (blue solid peak), staurosporine treated (green line)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

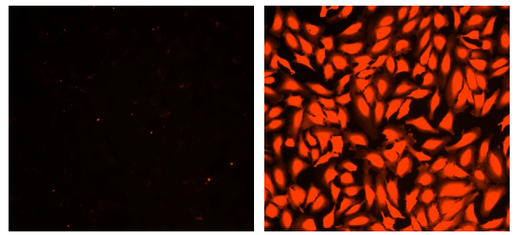

Figure 3.6. Images of HeLa cells stained with Cell Explorer™ Fixable Dead Cell Staining Kit (Cat# 22603) in a 96-well Costar black wall/clear bottom plate. Left: Live HeLa cells; Right: Fixed HeLa cells.

Table 3.4 Fixable Dead Cell Staining Kits

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
22600	Cell Explorer™ Fixable Dead Cell Staining Kit *Blue Fluorescence*	200 tests	353	442
22500	Cell Explorer™ Fixable Dead Cell Staining Kit *Blue Fluorescence with 405 nm Excitation*	200 tests	410	450
22604	Cell Explorer™ Fixable Dead Cell Staining Kit *Deep Red Fluorescence*	200 tests	649	660
22601	Cell Explorer™ Fixable Dead Cell Staining Kit *Green Fluorescence*	200 tests	498	521
22501	Cell Explorer™ Fixable Dead Cell Staining Kit *Green Fluorescence with 405 nm Excitation*	200 tests	408	512
22602	Cell Explorer™ Fixable Dead Cell Staining Kit *Orange Fluorescence*	200 tests	547	573
22502	Cell Explorer™ Fixable Dead Cell Staining Kit *Orange Fluorescence with 405 nm Excitation*	200 tests	398	550
22603	Cell Explorer™ Fixable Dead Cell Staining Kit *Red fluorescence*	200 tests	583	603

iFluor™ Labeling Probes

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

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

Key Features of iFluor™ Fluorescent Labeling Dyes

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

Table 4.1 iFluor™ Dye Equivalents of Common Dyes

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

Table 4.2 Amine-Reactive iFluor™ Dyes for Labeling Antibodies

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

Table 4.3 Thiol-Reactive iFluor™ Dyes for Labeling Antibodies

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

iFluor™ Dye-Labeled Antibodies

AAT Bioquest iFluor™ dyes are optimized for labeling proteins, in particular, antibodies. These dyes are bright, photostable and have minimal quenching on proteins. They can be well excited by the major laser lines of fluorescence instruments (e.g., 350, 405, 488, 555 and 633 nm). The almost identical spectral characteristics to those of Alexa Fluor® and DyLight™ make iFluor™-labeled secondary antibody conjugates an excellent alternative to the anti-IgG conjugates of Alexa Fluor® and DyLight™. Secondary antibodies bind to the primary antibody to assist in detection, sorting and purification of target antigens. Our secondary antibodies are used throughout various types of assays, including ELISA or Western Blot. The types of the secondary antibodies are selected according to the source of the primary antibody, the class of the primary antibody (e.g., IgG or IgM), and the kind of label which is preferred.

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Labeling Probes

Table 4.4 iFluor™ Dye-Labeled Secondary Antibodies

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

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iFluor™ Phalloidin Conjugates for Labeling F-actins

Actin is a globular, roughly 42-kDa protein found in almost all eukaryotic cells. It is also one of the most highly-conserved proteins, differing by no more than 20% in species as diverse as algae and humans. Actin is the monomeric subunit of two types of filaments in cells: microfilaments, one of the three major components of the cytoskeleton, and thin filaments, part of the contractile apparatus in muscle cells. Thus, actin participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, as well as the establishment and maintenance of cell junctions and cell shape. Phalloidin, a bicyclic heptapeptide toxin, binds specifically at the interface between F-actin subunits, locking adjacent subunits together. Phalloidin binds to actin filaments much more tightly than to actin monomers, leading to a decrease in the rate constant for the dissociation of actin subunits from filament ends, essentially stabilizing actin filaments through the prevention of filament depolymerization. Moreover, phalloidin is found to inhibit the ATP hydrolysis activity of F-actin. Thus, phalloidin traps actin monomers in a conformation distinct from G-actin and it stabilizes the structure of F-actin by greatly reducing the rate constant for monomer dissociation.

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

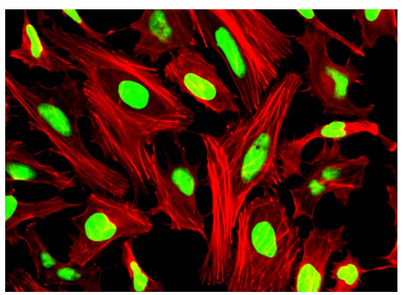

Figure 4.1. Image of HeLa cells. Actin filaments were stained with Phalloidin-iFluor™ 680 conjugate (red, Cat# 23128), and nuclei were stained with Nuclear Green™ DCS1 (green, Cat#17550).

Table 4.5 Phalloidin-iFluor™ Conjugates

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

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

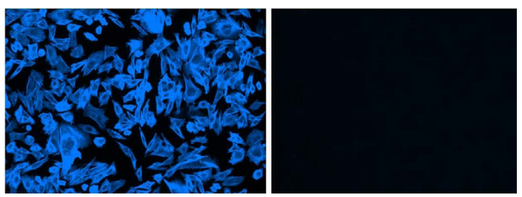

Figure 4.2. Images of fixed CPA cells stained with Cell Navigator™ F-actin Labeling Kit (Cat# 22660) in a 96-well Costar black wall/clear bottom plate A: Label the cells with 1X Phalloidin-iFluor™ 350 for 30 minutes only. B: Treat the cells with phalloidin for 10 minutes, then stain them with 1X Phalloidin-iFluor™ 350 for 30 minutes.

Table 4.6 F-actin Labeling Kits

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
22660	Cell Navigator™ F-actin Labeling Kit *Blue Fluorescence*	1 kit	353	442
22661	Cell Navigator™ F-actin Labeling Kit *Green Fluorescence*	1 kit	498	520
22663	Cell Navigator™ F-actin Labeling Kit *Orange Fluorescence*	1 kit	546	575
22664	Cell Navigator™ F-actin Labeling Kit *Red Fluorescence*	1 kit	583	603

trFluor™ Bioconjugates for Developing No Wash ELISA Assays

Time-Resolved Fluorescent Probes

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

Key Features of trFluor™ Dyes

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

Table 4.7 trFluor™ Labeling Dyes

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
1433	trFluor™ Eu Succinimidyl Ester	1 mg	346	617
1434	trFluor™ Eu Maleimide	100 µg	346	617
1443	trFluor™ Tb Succinimidyl Ester	1 mg	330	544
1444	trFluor™ Tb Maleimide	100 µg	330	544

trFluor™ Dye-Labeled Secondary Detection Reagents

trFluor™ bioconjugates comprise proteins (streptavidin or anti-IgGs) with trFluor™ dye covalently attached as the time-resolved fluorescent tag. They are commonly used as second step reagents for indirect immunofluorescent staining, when used in conjunction with primary antibodies. They are very valuable tools for biotin-developing biological assays and tests using TR-FRET platform. A variety of the complementary biotinylated reagents and primary antibodies are available from numerous commercial vendors.

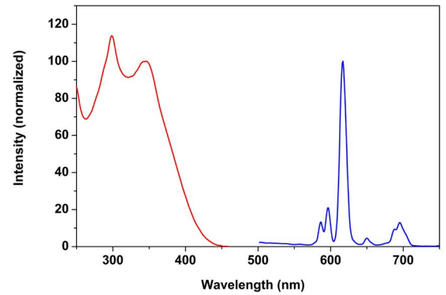

Figure 4.3. The excitation and emission spectra of trFluor™ Eu Goat Anti-Rabbit IgG conjugate (Cat# 16668) in PBS buffer (pH 7.2).

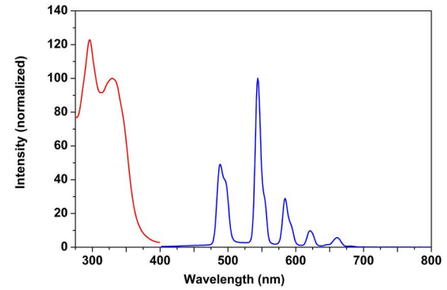

Figure 4.4. The excitation and emission spectra of trFluor™ Tb Goat Anti-Rabbit IgG conjugate (Cat# 16669) in PBS buffer (pH 7.2).

Table 4.8 trFluor™ Dye-Labeled Bioconjugates

Cat. #	Product Description	Size	Ex (nm)	Em (nm)
16518	trFluor™ Eu Goat Anti-Mouse IgG (H+L)	100 µg	346	617
16668	trFluor™ Eu Goat Anti-Rabbit IgG (H+L)	100 µg	346	617
16725	trFluor™ Eu Goat Anti-Rabbit IgG (H+L) *Cross Adsorbed*	100 µg	346	617
16925	trFluor™ Eu-streptavidin conjugate	100 µg	346	617
16519	trFluor™ Tb Goat Anti-Mouse IgG (H+L)	100 µg	330	544
16599	trFluor™ Tb Goat Anti-Mouse IgG (H+L) *Cross Adsorbed*	100 µg	330	544
16669	trFluor™ Tb Goat Anti-Rabbit IgG (H+L)	100 µg	330	544
16726	trFluor™ Tb Goat Anti-Rabbit IgG (H+L) *Cross Adsorbed*	100 µg	330	544
16926	trFluor™ Tb-Streptavidin Conjugate	100 µg	330	544

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