New Product Highlights
Monitoring Cell Cycles
Multicolor Labeling of Cell Nucleus with DNA-Binding Dyes
DAX-J2™ Ratio 580/460 for Detecting Nitric Oxide (NO)

Biochemical Assays
Neuraminidase Assay
Sphingomyelinase Assay
Multiplexing Caspase Activity Detection

Cell-Based Assays
Ratio Imaging of Cells with RatioWorks™ Probes
Multicolor Labeling of Dead Cells for Flow Cytometric Analysis

Labeling Probes
iFluor™ Fluorescent Probes for Labeling Antibodies
trFluor™ Bioconjugates for Developing No Wash ELISA Assays
From the President of AAT Bioquest

AAT Bioquest, Inc. (formerly ABD Bioquest, Inc.) develops, manufactures and markets biochemical 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

New Product Highlights

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 divide to 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.

The 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 from the dye is 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.

Table 1. Cell Cycle Assay Kits

<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Product Description</th>
<th>Size</th>
<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22841</td>
<td>Cell Meter™ Fluorimetric Cell Cycle Assay Kit <em>Green Fluorescence Optimized for Flow Cytometry</em></td>
<td>100 tests</td>
<td>503</td>
<td>526</td>
</tr>
<tr>
<td>22842</td>
<td>Cell Meter™ Fluorimetric Cell Cycle Assay Kit <em>Red Fluorescence Optimized for Flow Cytometry</em></td>
<td>100 tests</td>
<td>635</td>
<td>617</td>
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</table>

Biochemical Assays

Neuraminidase Assay 4
Sphingomyelinase Assay 4
DAX-J2™ Ratio 580/460 3

Cell-Based Assays

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Trademarks of Other Companies

AlkaFluor™ (Electrophoresis)
BODIPY™ (BD Biosciences)
C1™ (Cell HealthCare)
Delight™ (Thermo Fisher)
Fluo-4 (Molecular Probes)
GFP, EGFP and Cy5® (Belmont Healthcare)
Nuclear Green™ (BD Horizon)
Phalloidin™ (BD Biosciences)
ProTerra™ (BioAssay Systems)
Streptavidin™ (Molecular Probes)

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<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Product Description</th>
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<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22841</td>
<td>Cell Meter™ Fluorimetric Cell Cycle Assay Kit <em>Green Fluorescence Optimized for Flow Cytometry</em></td>
<td>100 tests</td>
<td>503</td>
<td>526</td>
</tr>
<tr>
<td>22842</td>
<td>Cell Meter™ Fluorimetric Cell Cycle Assay Kit <em>Red Fluorescence Optimized for Flow Cytometry</em></td>
<td>100 tests</td>
<td>635</td>
<td>617</td>
</tr>
</tbody>
</table>

Figure 1.2. DNA profile in growing and camptothecin treated Jarkut cells. Jarkut cells were treated without (red) or with 20 µM camptothecin (blue) at a 37°C, 5% CO2 incubator for about 8 hours, and assayed with Cell Meter™ Fluorimetric Cell Cycle Assay kit (EXP2014M). The fluorescence intensity of Nuclear Green™ LCS1 was measured with a FACsCalibur™ Flow cytometer using the FL1 channel. In growing Jarkut 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 Jarkut cells. Jarkut 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.
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 complexes 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 chromosomal organization.

**Labeling the Nuclei of Live Cells**

Nuclear Green™ LCS1, Nuclear Orange™ LCS1, Nuclear Red™ LCS1 and Nuclear Yellow are fluorescent, 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 dead, fixed or apoptotic cells. As 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 equipped with a 405 nm violet laser source. This DNA-binding dye might be useful for multicolor analysis of live cells with the filter sets of Pacific Blue™ and BD Horizon™ V450.

**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. The fluorescence 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.

**Table 1.2 Cell Nuclear Stains**

<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Product Description</th>
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<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17501</td>
<td>7-AAD [<em>7-Aminoactinomycin D</em>]</td>
<td>1 mg</td>
<td>546</td>
<td>647</td>
</tr>
<tr>
<td>17510</td>
<td>DAPI (4,6-Diamidino-2-phenylindole, dihydrochloride) <em>UltraPure Grade</em></td>
<td>10 mg</td>
<td>358</td>
<td>461</td>
</tr>
<tr>
<td>17562</td>
<td>Hoechst 33342 <em>UltraPure Grade</em></td>
<td>5 mg</td>
<td>518</td>
<td>600</td>
</tr>
<tr>
<td>17530</td>
<td>Hoechst 33342 <em>UltraPure Grade</em></td>
<td>100 mg</td>
<td>352</td>
<td>461</td>
</tr>
<tr>
<td>17537</td>
<td>Hoechst 33480 <em>UltraPure Grade</em></td>
<td>5 mg</td>
<td>368</td>
<td>437</td>
</tr>
<tr>
<td>17514</td>
<td>Hydroxybutyramidine</td>
<td>10 mg</td>
<td>360</td>
<td>625</td>
</tr>
<tr>
<td>17543</td>
<td>Nuclear Blue™ LCS1</td>
<td>0.5 mL</td>
<td>503</td>
<td>526</td>
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<tr>
<td>17550</td>
<td>Nuclear Green™ LCS1</td>
<td>0.5 mL</td>
<td>503</td>
<td>526</td>
</tr>
<tr>
<td>17540</td>
<td>Nuclear Green™ LCS1</td>
<td>0.5 mL</td>
<td>503</td>
<td>526</td>
</tr>
<tr>
<td>17551</td>
<td>Nuclear Orange™ LCS1</td>
<td>0.5 mL</td>
<td>528</td>
<td>576</td>
</tr>
<tr>
<td>17541</td>
<td>Nuclear Orange™ LCS1</td>
<td>0.5 mL</td>
<td>514</td>
<td>555</td>
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<tr>
<td>17552</td>
<td>Nuclear Red™ LCS1</td>
<td>0.5 mL</td>
<td>631</td>
<td>651</td>
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<tr>
<td>17542</td>
<td>Nuclear Red™ LCS1</td>
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<td>622</td>
<td>645</td>
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<tr>
<td>17539</td>
<td>Nuclear Yellow [Hoechst 3769121]</td>
<td>25 mg</td>
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<td>17515</td>
<td>Propidium Iodide <em>UltraPure Grade</em></td>
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**Table 1.3. Multicolor Nitric Oxide (NO) Detection Probes**

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

**DAX-J2™ Ratio 580/460**

Fluorescence response of DAX-J2™ Ratio 580/460 (2 µM) to different reactive oxygen species (ROS) in PBS buffer (pH 7.2). The fluorescence intensities were measured at 580 nm and 460 nm respectively.
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 in the mucociliary apparatus 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 0.1 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.

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 saposins, the lysosomal acidic SMases 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.

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.
Intracellular 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 into account 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 bypass these issues by observing emission or excitation wavelength shifts of fluorophores or by comparing the excitation or emission 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 upon calcium binding). Therefore, the lack of appropriate fluorescent probes for acidic organelles 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)methoxyphenyl)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 probe for acidic organelles with \( pK_a \) of 4.47. PDMPO selectively labels acid organelles (such as lyso-somes) 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 acidicotropic 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 BCEFl dextran conjugates 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

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Description</th>
<th>Size</th>
<th>Zero Calcium</th>
<th>High Calcium</th>
<th>( K_d ) (nM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>to 5 mg</td>
<td>Ex (nm)</td>
<td>Em (nm)</td>
<td>Ex (nm)</td>
</tr>
<tr>
<td>21034</td>
<td>BTC AM</td>
<td>1 mg</td>
<td>464</td>
<td>333</td>
<td>401</td>
</tr>
<tr>
<td>21053</td>
<td>BTC, tetrapotassium salt</td>
<td>1 mg</td>
<td>464</td>
<td>333</td>
<td>401</td>
</tr>
<tr>
<td>21021</td>
<td>Fura-2 AM <em>UltraPure grade</em></td>
<td>1 mg</td>
<td>363</td>
<td>512</td>
<td>335</td>
</tr>
<tr>
<td>21025</td>
<td>Fura-2, pentapotassium salt</td>
<td>1 mg</td>
<td>363</td>
<td>512</td>
<td>335</td>
</tr>
<tr>
<td>21026</td>
<td>Fura-2, pentasodium salt</td>
<td>1 mg</td>
<td>363</td>
<td>512</td>
<td>335</td>
</tr>
<tr>
<td>21058</td>
<td>Fura-8™, potassium salt</td>
<td>1 mg</td>
<td>386</td>
<td>532</td>
<td>354</td>
</tr>
<tr>
<td>21054</td>
<td>Fura-8™, sodium salt</td>
<td>1 mg</td>
<td>386</td>
<td>532</td>
<td>354</td>
</tr>
<tr>
<td>21032</td>
<td>Indo-1 AM <em>UltraPure grade</em></td>
<td>1 mg</td>
<td>346</td>
<td>475</td>
<td>330</td>
</tr>
<tr>
<td>21040</td>
<td>Indo-1, pentapotassium salt</td>
<td>1 mg</td>
<td>346</td>
<td>475</td>
<td>330</td>
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<td>21044</td>
<td>Indo-1, pentasodium salt</td>
<td>1 mg</td>
<td>346</td>
<td>475</td>
<td>330</td>
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<tr>
<td>21050</td>
<td>Quin-2 AM 5 mg</td>
<td>1 mg</td>
<td>353</td>
<td>495</td>
<td>333</td>
</tr>
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<td>21052</td>
<td>Quin-2, tetrapotassium salt</td>
<td>5 mg</td>
<td>353</td>
<td>495</td>
<td>333</td>
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</table>

Key Features of Fura-8™ Calcium Indicator

- **Fura-8™ responds to calcium in 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 3.2. ATP dose responses in CHO-K1 cells measured with Fura-2 AM (Cat# 21021) and Fura 8 AM (Cat# 21051) 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 exciliated with Fura-2 AM or Fura 8 AM 15 min prior to ATP loading solution for 1 hr at room temperature. ATP (50 μM) was added by FluorBrite®

Figures 3.1 and 3.2, Fura-8™ AM is more sensitive to calcium than Fluo-8® and Cal-520™. AAT Bioquest has recently developed Fura-8™ AM, a sensitive, yet specific, ratiometric calcium indicator, it has certain limitations, e.g., lower sensitivity compared to the single wavelength calcium dyes such as Fura-2 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 also be used for flow cytometric analysis of calcium in cells due to its excellent excitation at 465 nm of violet laser.

Fluo-8® and new CCD cameras allows affordable quantitative and fast signal detection. The recent development of ultrafast imaging setup. However, ratiometric imaging depends on a fast intracellular ion, voltage and 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.
fluorescence microscopy or flow cytometry. The pH in lysosomes can be measured with PDMPO dextrans using fluorescence emission occurs near the pKa of the dye at pH ~4.2. Endosomes to yellow in the acidic lysosomes. The greatest change taken up by the cells and moves through the endocytic pathway, 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 consistent with quite a few different isomers.

**Table 3.2 Reactive Fluorescent pH Probes and Their Dextran Conjugate**

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Description</th>
<th>Size</th>
<th>pKa</th>
<th>Ex (nm)</th>
<th>Em (nm)</th>
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<tbody>
<tr>
<td>21217</td>
<td>Potex™ Green 500 Dextran</td>
<td>1 mg</td>
<td>7.0</td>
<td>503</td>
<td>528</td>
</tr>
<tr>
<td>21216</td>
<td>Potex™ Green 500 SE</td>
<td>1 mg</td>
<td>643</td>
<td>505</td>
<td></td>
</tr>
<tr>
<td>21209</td>
<td>Potex™ Red 640/Latex Bead Conjugate</td>
<td>1 ud</td>
<td>575</td>
<td>597</td>
<td></td>
</tr>
<tr>
<td>21208</td>
<td>Potex™ Red 640, SE</td>
<td>1 mg</td>
<td>575</td>
<td>597</td>
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<tr>
<td>21210</td>
<td>RatioWorks™ PDMPO, SE</td>
<td>1 mg</td>
<td>405</td>
<td>550</td>
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<tr>
<td>21211</td>
<td>RatioWorks™ PDMPO Dextran</td>
<td>1 mg</td>
<td>405</td>
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**Table 3.3 Fluorescent pH Probes for Near-Neutral pH Environments**

<table>
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<tr>
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<th>Product Description</th>
<th>Size</th>
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<td>1 mg</td>
<td>7.0</td>
<td>503</td>
<td>528</td>
</tr>
<tr>
<td>21202</td>
<td>BCECF AM</td>
<td>1 mg</td>
<td>7.0</td>
<td>503</td>
<td>528</td>
</tr>
<tr>
<td>21203</td>
<td>BCECF AM “Ultra Pure Grade”</td>
<td>250 µg</td>
<td>7.0</td>
<td>503</td>
<td>528</td>
</tr>
<tr>
<td>21200</td>
<td>Cell-Tracker™ Fluorescent Intracellular pH Assay Kit</td>
<td>1000 tests</td>
<td>N/A</td>
<td>503</td>
<td>528</td>
</tr>
<tr>
<td>21207</td>
<td>RatioWorks™ BCECF AM “Super Replacement to BCECF”</td>
<td>1 mg</td>
<td>7.0</td>
<td>503</td>
<td>528</td>
</tr>
<tr>
<td>21209</td>
<td>RatioWorks™ BCECF AM “Super Replacement to BCECF”</td>
<td>1 mg</td>
<td>7.0</td>
<td>503</td>
<td>528</td>
</tr>
</tbody>
</table>

**Table 3.4 Fixable Dead Cell Staining Kits**

<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Product Description</th>
<th>Size</th>
<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22600</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Blue Fluorescence”</td>
<td>200 tests</td>
<td>353</td>
<td>442</td>
</tr>
<tr>
<td>22500</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Blue Fluorescence with 405 nm Excitation”</td>
<td>200 tests</td>
<td>410</td>
<td>450</td>
</tr>
<tr>
<td>22604</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Deep Red Fluorescence”</td>
<td>200 tests</td>
<td>649</td>
<td>660</td>
</tr>
<tr>
<td>22601</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Green Fluorescence”</td>
<td>200 tests</td>
<td>498</td>
<td>521</td>
</tr>
<tr>
<td>22501</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Green Fluorescence with 405 nm Excitation”</td>
<td>200 tests</td>
<td>408</td>
<td>512</td>
</tr>
<tr>
<td>22602</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Orange Fluorescence”</td>
<td>200 tests</td>
<td>547</td>
<td>573</td>
</tr>
<tr>
<td>22502</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Orange Fluorescence with 405 nm Excitation”</td>
<td>200 tests</td>
<td>398</td>
<td>550</td>
</tr>
<tr>
<td>22603</td>
<td>Cell Explorer™ Fixable Dead Cell Staining Kit “Red Fluorescence”</td>
<td>200 tests</td>
<td>583</td>
<td>603</td>
</tr>
</tbody>
</table>
iFluor™ Labeling Probes

AAT Bioquest is rapidly expanding our product lines to meet your research done with less money.

**iFluor™ Dyes for Labeling Antibodies**

- Excellent water solubility
- Available in a variety of fluorescence colors
- Their conjugates exhibit more intense fluorescence than other similar conjugates of Alexa Fluor® and DyLight™.
- Absorption spectra match the principal output wavelengths of common excitation sources.
- New research with less money.

**Table 4.1 iFluor™ Dye Equivalents of Common Dyes**

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Size Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexa Fluor® 350</td>
<td>345</td>
<td>442</td>
</tr>
<tr>
<td>Alexa Fluor® 405</td>
<td>401</td>
<td>420</td>
</tr>
<tr>
<td>Alexa Fluor® 488</td>
<td>491</td>
<td>514</td>
</tr>
<tr>
<td>Alexa Fluor® 555</td>
<td>559</td>
<td>569</td>
</tr>
<tr>
<td>Alexa Fluor® 594</td>
<td>592</td>
<td>614</td>
</tr>
<tr>
<td>Alexa Fluor® 633</td>
<td>638</td>
<td>655</td>
</tr>
<tr>
<td>Alexa Fluor® 750</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>Alexa Fluor® 700</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>Alexa Fluor® 790</td>
<td>753</td>
<td>779</td>
</tr>
</tbody>
</table>

**Table 4.2 Amine- Reactive iFluor™ Dyes for Labeling Antibodies**

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Size Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iFluor™ 488 Maleimide</td>
<td>345</td>
<td>442</td>
</tr>
<tr>
<td>iFluor™ 647 Maleimide</td>
<td>491</td>
<td>514</td>
</tr>
<tr>
<td>iFluor™ 633 Maleimide</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>iFluor™ 680 Maleimide</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>iFluor™ 700 Maleimide</td>
<td>693</td>
<td>713</td>
</tr>
<tr>
<td>iFluor™ 790 Maleimide</td>
<td>753</td>
<td>779</td>
</tr>
</tbody>
</table>

**Table 4.3 Thiols- Reactive iFluor™ Dyes for Labeling Antibodies**

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Size Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iFluor™ 488 Succinimidyl Ester</td>
<td>345</td>
<td>442</td>
</tr>
<tr>
<td>iFluor™ 647 Succinimidyl Ester</td>
<td>491</td>
<td>514</td>
</tr>
<tr>
<td>iFluor™ 633 Succinimidyl Ester</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>iFluor™ 680 Succinimidyl Ester</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>iFluor™ 700 Succinimidyl Ester</td>
<td>693</td>
<td>713</td>
</tr>
<tr>
<td>iFluor™ 790 Succinimidyl Ester</td>
<td>753</td>
<td>779</td>
</tr>
</tbody>
</table>

**Table 4.4 iFluor™ Dye-Labeled Secondary Antibodies**

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Size Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iFluor™ 350 Goat Anti-Mouse IgG (H+L)</td>
<td>345</td>
<td>442</td>
</tr>
<tr>
<td>iFluor™ 405 Goat Anti-Mouse IgG (H+L)</td>
<td>401</td>
<td>420</td>
</tr>
<tr>
<td>iFluor™ 488 Goat Anti-Mouse IgG (H+L)</td>
<td>491</td>
<td>514</td>
</tr>
<tr>
<td>iFluor™ 555 Goat Anti-Mouse IgG (H+L)</td>
<td>559</td>
<td>569</td>
</tr>
<tr>
<td>iFluor™ 594 Goat Anti-Mouse IgG (H+L)</td>
<td>592</td>
<td>614</td>
</tr>
<tr>
<td>iFluor™ 633 Goat Anti-Mouse IgG (H+L)</td>
<td>638</td>
<td>655</td>
</tr>
<tr>
<td>iFluor™ 647 Goat Anti-Mouse IgG (H+L)</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>iFluor™ 680 Goat Anti-Mouse IgG (H+L)</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>iFluor™ 700 Goat Anti-Mouse IgG (H+L)</td>
<td>693</td>
<td>713</td>
</tr>
<tr>
<td>iFluor™ 790 Goat Anti-Mouse IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>iFluor™ 730 Goat Anti-Mouse IgG (H+L)</td>
<td>782</td>
<td>811</td>
</tr>
<tr>
<td>iFluor™ 750 Goat Anti-Mouse IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>iFluor™ 700 Goat Anti-Rabbit IgG (H+L)</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>iFluor™ 693 Goat Anti-Rabbit IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>iFluor™ 638 Goat Anti-Rabbit IgG (H+L)</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>iFluor™ 647 Goat Anti-Rabbit IgG (H+L)</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>iFluor™ 633 Goat Anti-Rabbit IgG (H+L)</td>
<td>693</td>
<td>713</td>
</tr>
<tr>
<td>iFluor™ 555 Goat Anti-Rabbit IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>iFluor™ 782 Goat Anti-Rabbit IgG (H+L)</td>
<td>782</td>
<td>811</td>
</tr>
<tr>
<td>iFluor™ 700 Goat Anti-Rabbit IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>iFluor™ 682 Goat Anti-Rabbit IgG (H+L)</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>iFluor™ 638 Goat Anti-Rabbit IgG (H+L)</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>iFluor™ 647 Goat Anti-Rabbit IgG (H+L)</td>
<td>693</td>
<td>713</td>
</tr>
<tr>
<td>iFluor™ 633 Goat Anti-Rabbit IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>iFluor™ 555 Goat Anti-Rabbit IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>iFluor™ 782 Goat Anti-Rabbit IgG (H+L)</td>
<td>753</td>
<td>779</td>
</tr>
</tbody>
</table>

**If you are using Try this iFluor™ dye**

| AlexaFlour® 350, 488, 568, 635 | iFluor™ 350 |
| AlexaFlour® 405, 488, 568, 635 | iFluor™ 405 |
| AlexaFlour® 488, 568, 635, 647 | iFluor™ 488 |
| AlexaFlour® 514, 568, 635, 647 | iFluor™ 514 |
| AlexaFlour® 532, 568, 635, 647 | iFluor™ 532 |
| AlexaFlour® 555, 568, 635, 647 | iFluor™ 555 |
| AlexaFlour® 594, 568, 635, 647 | iFluor™ 594 |
| AlexaFlour® 633, 568, 635, 647 | iFluor™ 633 |
| AlexaFlour® 647, 568, 635, 647 | iFluor™ 647 |
| AlexaFlour® 680, 568, 635, 647 | iFluor™ 680 |
| AlexaFlour® 700, 568, 635, 647 | iFluor™ 700 |
| AlexaFlour® 790, 568, 635, 647 | iFluor™ 790 |

**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 lasers of fluorescence instruments (e.g., 350, 405, 488, 555 and 633 nm). The almost identical spectral characteristics to those of AlexaFlour® and DyLight™ make iFluor™-labeled secondary antibody conjugates an excellent alternative to the anti-IgG conjugates of AlexaFlour® 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.
iFluor™ Phalloidin Conjugates for Labeling F-actin

Phalloidin is a globular, roughly 42kDa 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 to actin filaments at high resolution. Used at nanomolar concentrations, phalloidin functions differently at actin networks in fixed cells. The kits use fluorescent phalloidin conjugates that are selectively bound to F-actins. The fluorescent phalloidin conjugates have large Stokes shifts and extremely high sensitivity.

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 have large Stokes shifts and extremely high sensitivity. The kits provide all the essential components with an optimized labeling protocol.

Table 4.5 Phalloidin-iFluor Conjugates

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Description</th>
<th>Size (mm)</th>
<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23100</td>
<td>Phalloidin Alexa 488 Conjugate</td>
<td>300 tests</td>
<td>486</td>
<td>522</td>
</tr>
<tr>
<td>23101</td>
<td>Phalloidin Alexa 568 Conjugate</td>
<td>300 tests</td>
<td>565</td>
<td>601</td>
</tr>
<tr>
<td>23102</td>
<td>Phalloidin Alexa 647 Conjugate</td>
<td>300 tests</td>
<td>645</td>
<td>680</td>
</tr>
<tr>
<td>23103</td>
<td>Phalloidin Alexa 633 Conjugate</td>
<td>300 tests</td>
<td>633</td>
<td>669</td>
</tr>
<tr>
<td>23104</td>
<td>Phalloidin Alexa 594 Conjugate</td>
<td>300 tests</td>
<td>594</td>
<td>629</td>
</tr>
<tr>
<td>23105</td>
<td>Phalloidin Alexa 514 Conjugate</td>
<td>300 tests</td>
<td>514</td>
<td>549</td>
</tr>
<tr>
<td>23106</td>
<td>Phalloidin Alexa 488 Conjugate</td>
<td>300 tests</td>
<td>488</td>
<td>523</td>
</tr>
</tbody>
</table>

trFluor™ Bioconjugates for Developing No Wash ELISA Assays

Many biological compounds present in cells, serum or other biological fluids are naturally fluorescent, and thus the use of conventional, prompt fluorescence 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 fluorescent dyes like 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.

Table 4.7 trFluor™ Dye-Labeled Bioconjugates

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Description</th>
<th>Size (mm)</th>
<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16516</td>
<td>trFluor™ Tb Anti-Rabbit IgG (H+L) Adsorbed* 100 μg</td>
<td>100 μg</td>
<td>346</td>
<td>617</td>
</tr>
<tr>
<td>16517</td>
<td>trFluor™ Tb Anti-Mouse IgG (H+L) Adsorbed* 100 μg</td>
<td>100 μg</td>
<td>330</td>
<td>546</td>
</tr>
<tr>
<td>16518</td>
<td>trFluor™ Tb Anti-Guinea Pig IgG (H+L) Adsorbed* 100 μg</td>
<td>100 μg</td>
<td>330</td>
<td>546</td>
</tr>
</tbody>
</table>

trFluor™ Dye-Labeled Secondary Detection Reagents

trFluor™ bioconjugates comprise proteins (streptavidin or anti-IgG) 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 bioimaging and developing biological assays and tests using TR-FRET platform.