New Product Highlights
Ratiometric Fura-6™ Calcium Indicators
Non-Radioactive Glucose Uptake Assays
Multicolor Cell Viability Assays for Flow Cytometry

Biochemical Assays
Colorimetric Quantitation of Ammonia
Fluorimetric Quantitation of Formaldehyde
Fluorimetric Quantitation of Lactate Dehydrogenase
Fluorimetric Measurement of Glucose-6-Phosphate
Detection of Reactive Oxygen Species (ROS)

Cell-Based Assays
Fluo-8® Green Calcium Indicators
Gaussia Luciferase Reporter Gene Assay
Firefly Luciferase Reporter Gene Assay
iFluor™ 594, a Bright Fluorescent Labeling Dye

Labeling Probes
trFluor™ Eu TRF Labeling Dyes
trFluor™ Tb TRF Labeling Dyes
ReadiLink™ iFluor™ Antibody Labeling Kits
Table of Contents

New Product Highlights
- Ratiometric Fura-6™ Calcium Indicators 1
- Non-Radioactive Glucose Uptake Assays 2
- Multicolor Cell Viability Assays for Flow Cytometry 3

Biochemical Assays
- Colorimetric Quantitation of Ammonia 4
- Fluorimetric Quantitation of Formaldehyde 4
- Fluorimetric Quantitation of Lactate Dehydrogenase 5
- Fluorimetric Measurement of Glucose-6-Phosphate 5
- Detection of Reactive Oxygen Species (ROS) 6

Cell-Based Assays
- Fluo-8® Green Calcium Indicators 7
- Gaussia Luciferase Reporter Gene Assay 9
- Firefly Luciferase Reporter Gene Assay 9
- iFluor™ 594, a Bright Fluorescent Labeling Dye 10

Labeling Probes
- trFluor™ Eu TRF Labeling Dye 12
- trFluor™ Tb TRF Labeling Dye 12
- ReadiLink™ iFluor™ Antibody Labeling Kits 13

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

Trademarks of AAT Bioquest
- AAT Bioquest™
- Amplite™
- Cal-520™
- Cell Explorer™
- Fluo-8™
- Fluo-8F™
- Fluo-8H™
- Fluo-8L™
- Fura-6™
- iFluor™
- mFluor™
- ReadiLink™
- ReadiView™
- ROS Brite™
- Screen Quest™
- Tide Quencher™
- trFluor™

Trademarks of Other Companies
- Alexa Fluor® (Invitrogen)
- Cy5® (GE Healthcare)
- DyLight™ (ThermoFisher)
- FlexStation® (Molecular Devices)
- SpectrumMax® (Molecular Devices)
Ratiometric Fura-6™ Calcium Indicators

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-6™ to improve the calcium response of Fura-2. As demonstrated in Figures 1.1 and 1.2, Fura-6™ AM is more sensitive to calcium than Fura-2 AM. In addition, Fura-6™ has its emission shifted to longer wavelength (Em = 525 nm). Fura-6™ might be also used for the flow cytometric analysis of calcium in cells due to its excellent excitation at 405 nm that perfectly matches the violet laser line of flow cytometer.

![Fluorescence excitation spectra of Fura-6™ in the presence of 0 to 39 µM free Ca2+](image)

**Figure 1.1.** Fluorescence excitation spectra of Fura-6™ in the presence of 0 to 39 µM free Ca2+.

**Table 1.1. Product Ordering Information**

<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Product Description</th>
<th>Size</th>
<th>Zero Calcium</th>
<th>High Calcium</th>
<th>Κₐ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ex (nm)</td>
<td>Em (nm)</td>
<td>Ex (nm)</td>
</tr>
<tr>
<td>21054</td>
<td>BTC AM</td>
<td>1 mg</td>
<td>464 533</td>
<td>401 529</td>
<td>7,000</td>
</tr>
<tr>
<td>21053</td>
<td>BTC, tetrapotassium salt</td>
<td>1 mg</td>
<td>464 533</td>
<td>401 529</td>
<td>7,000</td>
</tr>
<tr>
<td>21021</td>
<td>Fura-2 AM <em>UltraPure grade</em></td>
<td>1 mg</td>
<td>363 512</td>
<td>335 505</td>
<td>145</td>
</tr>
<tr>
<td>21025</td>
<td>Fura-2, pentapotassium salt</td>
<td>1 mg</td>
<td>363 512</td>
<td>335 505</td>
<td>145</td>
</tr>
<tr>
<td>21026</td>
<td>Fura-2, pentasodium salt</td>
<td>1 mg</td>
<td>363 512</td>
<td>335 505</td>
<td>145</td>
</tr>
<tr>
<td>21055</td>
<td>Fura-6™ AM</td>
<td>1 mg</td>
<td>386 532</td>
<td>354 524</td>
<td>260</td>
</tr>
<tr>
<td>21056</td>
<td>Fura-6™ AM</td>
<td>10x50 µg</td>
<td>386 532</td>
<td>354 524</td>
<td>260</td>
</tr>
<tr>
<td>21057</td>
<td>Fura-6™, potassium salt</td>
<td>1 mg</td>
<td>386 532</td>
<td>354 524</td>
<td>260</td>
</tr>
<tr>
<td>21058</td>
<td>Fura-6™, sodium salt</td>
<td>1 mg</td>
<td>386 532</td>
<td>354 524</td>
<td>260</td>
</tr>
<tr>
<td>21032</td>
<td>Indo-1 AM <em>UltraPure grade</em></td>
<td>1 mg</td>
<td>346 475</td>
<td>330 401</td>
<td>230</td>
</tr>
<tr>
<td>21040</td>
<td>Indo-1, pentapotassium salt</td>
<td>1 mg</td>
<td>346 475</td>
<td>330 401</td>
<td>230</td>
</tr>
<tr>
<td>21044</td>
<td>Indo-1, pentasodium salt</td>
<td>1 mg</td>
<td>346 475</td>
<td>330 401</td>
<td>230</td>
</tr>
<tr>
<td>21050</td>
<td>Quin-2 AM</td>
<td>1 mg</td>
<td>353 495</td>
<td>333 495</td>
<td>60</td>
</tr>
<tr>
<td>21052</td>
<td>Quin-2, tetrapotassium salt</td>
<td>5 mg</td>
<td>353 495</td>
<td>333 495</td>
<td>60</td>
</tr>
</tbody>
</table>

Key Features of Fura-6™

- Fura-6™ 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 1.2.** ATP dose responses in CHO-K1 cells measured with Fura-2 AM and Fura-6™ AM 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 and Fura-6™ AM calcium assay dye-loading solution respectively for 1 hour at room temperature. ATP (50 µL/well) was added by FlexStation®.
Glucose transport systems are responsible for transporting glucose across cell membranes. Measuring the uptake of 2-deoxyglucose (2-DG), a glucose analog, is widely accepted as a reliable method to estimate the amount of glucose uptake and to investigate the regulation of glucose metabolism and insulin resistance. The 2-DG uptake is commonly determined using the non-metabolized 2-DG labeled with tritium or C14. However, the use of a radiolabeled probe is costly and requires a tedious special handling procedure.

AAT Bioquest’s Screen Quest™ Colorimetric Glucose Uptake Assay Kit provides a sensitive and non-radioactive glucose uptake assay. The accumulated 2-DG6P accumulates in cells and is proportional to glucose uptake. The accumulated 2-DG6P is enzymatically coupled to generate NADPH, which is specifically monitored by a fluorogenic NADPH sensor. The signal can be read using an absorption microplate reader by reading the OD ratio of 570 nm to 610 nm.

Based on the same principle, AAT Bioquest’s Screen Quest™ Fluorimetric Glucose Uptake Assay Kit provides an even more sensitive and non-radioactive glucose uptake assay in tissues or cultured cells. The accumulated 2-DG6P is enzymatically coupled to generate NADPH, which is specifically monitored by a fluorogenic NADPH sensor. The signal can be read using a fluorescence microplate reader at Ex/Em = 540 nm/590 nm.

### Non-Radioactive Glucose Uptake Assays

- **Table 1.2. Product Ordering Information**

<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>11300</td>
<td>Amplite™ Fluorimetric Glucose Oxidase Assay Kit <em>Red Fluorescence</em></td>
<td>1 kit</td>
<td>571</td>
<td>585</td>
</tr>
<tr>
<td>40005</td>
<td>Amplite™ Glucose Quantitation Kit</td>
<td>1 kit</td>
<td>571</td>
<td>585</td>
</tr>
<tr>
<td>11705</td>
<td>Glucose-UDP-Fluorescein Conjugate</td>
<td>100 μg</td>
<td>490</td>
<td>514</td>
</tr>
<tr>
<td>11706</td>
<td>Glucose-UDP-(PEG)6-Fluorescein Conjugate</td>
<td>100 μg</td>
<td>490</td>
<td>514</td>
</tr>
<tr>
<td>36503</td>
<td>Screen Quest™ Colorimetric Glucose Uptake Assay Kit</td>
<td>1 kit</td>
<td>575</td>
<td>N/A</td>
</tr>
<tr>
<td>36500</td>
<td>Screen Quest™ Fluorimetric Glucose Uptake Assay Kit</td>
<td>1 kit</td>
<td>571</td>
<td>585</td>
</tr>
</tbody>
</table>

- **Table 1.3. Product Ordering Information**

<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 Cell Viability Assay Kit <em>Blue Fluorescence</em></td>
<td>1 kit</td>
<td>353</td>
<td>442</td>
</tr>
<tr>
<td>22500</td>
<td>Cell Explorer™ Fixable Cell Viability Assay Kit <em>Blue Fluorescence with 405 nm Excitation</em></td>
<td>1 kit</td>
<td>410</td>
<td>450</td>
</tr>
<tr>
<td>22604</td>
<td>Cell Explorer™ Fixable Cell Viability Assay Kit <em>Deep Red Fluorescence</em></td>
<td>1 kit</td>
<td>649</td>
<td>660</td>
</tr>
<tr>
<td>22601</td>
<td>Cell Explorer™ Fixable Cell Viability Assay Kit <em>Green Fluorescence</em></td>
<td>1 kit</td>
<td>498</td>
<td>521</td>
</tr>
<tr>
<td>22501</td>
<td>Cell Explorer™ Fixable Cell Viability Assay Kit <em>Green Fluorescence with 405 nm Excitation</em></td>
<td>1 kit</td>
<td>408</td>
<td>512</td>
</tr>
<tr>
<td>22602</td>
<td>Cell Explorer™ Fixable Cell Viability Assay Kit <em>Orange Fluorescence</em></td>
<td>1 kit</td>
<td>547</td>
<td>573</td>
</tr>
<tr>
<td>22502</td>
<td>Cell Explorer™ Fixable Cell Viability Assay Kit <em>Orange Fluorescence with 405 nm Excitation</em></td>
<td>1 kit</td>
<td>398</td>
<td>550</td>
</tr>
<tr>
<td>22603</td>
<td>Cell Explorer™ Fixable Cell Viability Assay Kit <em>Red Fluorescence</em></td>
<td>1 kit</td>
<td>583</td>
<td>603</td>
</tr>
</tbody>
</table>

- **Figure 1.4.** 2-DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with Screen Quest™ Fluorimetric Glucose Uptake Assay Kit (Cat# 36500) in a black wall/clear bottom cell culture Poly-D-Lysine plate using a Gemini (Molecular Devices) microplate reader. A: Negative Control, no insulin and no 2-DG treatment; B: 2-DG uptake in the absence of insulin; C: 2-DG uptake in the presence of 1 μM insulin; D: 2-DG uptake in the presence of 1 μM insulin and 200 μM phloretin; E: 2-DG uptake in the presence of 1 μM insulin and 1 mM D-glucose.

- **Figure 1.5.** Image of CHO cells fixed with formaldehyde and stained with Cell Explorer™ Fixable Cell Viability Assay Kit (Cat# 22601) in a Costar 96-well black well/clear bottom plate.

- **Figure 1.6.** Jurkat cells were treated and stained with Cell Explorer™ Fixable Cell Viability Assay Kits 22600 (Panel A) and 22604 (Panel B). The cells were fixed in 3.7% formaldehyde and analyzed by flow cytometry. Live (blue solid peak), staurosporine treated (green line) and heat-treated (red solid peak) cells were distinguished with Ex/Em = 488 nm/520 nm (FL1) channel (Panel A) and 633 nm/660 nm (FL4) channel (Panel B). Nearly identical results were obtained using unfixed cells.
Colorimetric Quantitation of Ammonia

Ammonia is an important source of nitrogen for living systems. It is produced in liver and converted to urea through the urea cycle. Ammonia is synthesized through amino acid metabolism and is toxic when present at high concentrations. Elevated levels of ammonia in blood (hyperammonemia) have been found in liver dysfunction (cirrhosis), while hyperammonemia is associated with defects in the urea cycle enzymes (e.g. ornithine transcarbamylase). The determination of ammonia is very useful for clinical laboratory to monitor health status.

AAT Bioquest’s Amplite™ Colorimetric Ammonia Quantitation Kit provides a simple and sensitive colorimetric method for the quantitation of ammonia in foods and biological samples such as serum, plasma and urine, etc. The assay is based on an enzyme-coupled reaction of ammonia, which generates a blue colored product. The absorbance of the blue product is proportional to the concentration of ammonia, which can be measured colorimetrically at 660-670 nm. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

Formaldehyde is a naturally occurring substance. Natural processes in the upper atmosphere may contribute up to 90 percent of the total formaldehyde in the environment. Formaldehyde, as well as its oligomers and hydrates are rarely encountered in living organisms. Methanogenesis proceeds via the equivalent of formaldehyde, but this one-carbon species is masked as a methylene group in methanopterin. Formaldehyde is the primary cause of methanol’s toxicity, since methanol is metabolized into toxic formaldehyde by alcohol dehydrogenase.

Fluorimetric Quantitation of Formaldehyde

AAT Bioquest’s Amplite™ Fluorimetric Formaldehyde Quantitation Kit is used for quantifying formaldehyde. The Kit uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with formaldehyde. This fluorimetric kit provides a sensitive mix-and-read method to detect formaldehyde. 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. Its signal can be easily read using a fluorescence microplate reader at Ex/Em = 400/510 nm.

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyzes the interconversion of pyruvate and lactate. Localized in the cytosol, LDH is present in a wide variety of organisms, including animals and plants. Cells release LDH into the bloodstream after tissue damage or red blood cell hemolysis. Since LDH is a fairly stable enzyme, it is widely used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has a broad range of applications.

AAT Bioquest’s Amplite™ Lactate Dehydrogenase Assay Kits provide both fluorimetric- and absorbance-based methods for detecting either L-lactate dehydrogenase (L-LDH) or D-lactate dehydrogenase (D-LDH). In the enzyme coupled assay, LDH is proportionally related to the concentration of NADH that is specifically monitored by a fluorogenic NADH sensor. The fluorescence signal can be read using a fluorescence microplate reader at Ex/Em = 540 nm/590 nm. We were able to detect as low as 1 mU/mL L-LDH in a 100 μL reaction volume. The assays are robust, and can be readily adapted for a wide variety of applications that require the measurement of L-LDH.

Fluorimetric Measurement of Glucose-6-Phosphate

Glucose-6-phosphate (G6P) is a key intermediate for glucose transport into cells. G6P may also be converted to glycogen or starch for storage in the liver and muscles. G6P is utilized by glucose-6-phosphate dehydrogenase (G6PD) to generate the reducing equivalents in the form of NADPH. This is particularly important in red blood cells where G6PD deficiency leads to hemolytic anemia.

AAT Bioquest’s Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit provides a simple, sensitive and rapid fluorescence-based method for detecting G6P in biological samples such as serum, plasma, urine, as well as in cell and tissue samples. In the coupled enzyme assay, the G6P concentration is proportionally related to NADPH that is specifically monitored by a fluorogenic NADPH sensor. The fluorescence signal can be read. A fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (Ex/Em = 540 nm/590 nm is recommended). With the Amplite™ G6P Assay Kit, we were able to detect as low as 0.3 μM G6P in a 100 μL reaction volume.

Table 2.1. Product Ordering Information

<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>10051</td>
<td>Amplite™ Colorimetric Aldehyde Quantitation Kit</td>
<td>1 kit</td>
<td>550</td>
<td>N/A</td>
</tr>
<tr>
<td>10053</td>
<td>Amplite™ Colorimetric Aldehyde Quantitation Kit <em>Blue Color</em></td>
<td>1 kit</td>
<td>620</td>
<td>N/A</td>
</tr>
<tr>
<td>10059</td>
<td>Amplite™ Colorimetric Ammonia Quantitation Kit</td>
<td>1 kit</td>
<td>650</td>
<td>N/A</td>
</tr>
<tr>
<td>10058</td>
<td>Amplite™ Colorimetric Urea Quantitation Kit <em>Blue Color</em></td>
<td>1 kit</td>
<td>650</td>
<td>N/A</td>
</tr>
<tr>
<td>10052</td>
<td>Amplite™ Fluorimetric Aldehyde Quantitation Kit</td>
<td>1 kit</td>
<td>360</td>
<td>450</td>
</tr>
<tr>
<td>10057</td>
<td>Amplite™ Fluorimetric Formaldehyde Quantitation Kit <em>Green Fluorescence</em></td>
<td>1 kit</td>
<td>400</td>
<td>510</td>
</tr>
</tbody>
</table>

Table 2.2. Product Ordering Information

<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>13809</td>
<td>Amplite™ Colorimetric D-Lactate Dehydrogenase (LDH) Assay Kit</td>
<td>1 kit</td>
<td>575</td>
<td>N/A</td>
</tr>
<tr>
<td>13807</td>
<td>Amplite™ Colorimetric Glucose-6-Phosphate Dehydrogenase (G6PD) Assay Kit</td>
<td>1 kit</td>
<td>575</td>
<td>N/A</td>
</tr>
<tr>
<td>13813</td>
<td>Amplite™ Colorimetric L-Lactate Dehydrogenase (LDH) Assay Kit</td>
<td>1 kit</td>
<td>575</td>
<td>N/A</td>
</tr>
<tr>
<td>13808</td>
<td>Amplite™ Fluorimetric D-Lactate Dehydrogenase (LDH) Assay Kit</td>
<td>1 kit</td>
<td>571</td>
<td>S55</td>
</tr>
<tr>
<td>13804</td>
<td>Amplite™ Fluorimetric Glucose-6-Phosphate Assay Kit</td>
<td>1 kit</td>
<td>571</td>
<td>S55</td>
</tr>
<tr>
<td>13806</td>
<td>Amplite™ Fluorimetric Glucose-6-Phosphate Dehydrogenase (G6PD) Assay Kit</td>
<td>1 kit</td>
<td>571</td>
<td>S55</td>
</tr>
<tr>
<td>13812</td>
<td>Amplite™ Fluorimetric L-Lactate Dehydrogenase (LDH) Assay Kit</td>
<td>1 kit</td>
<td>571</td>
<td>S55</td>
</tr>
</tbody>
</table>
Detection of Reactive Oxygen Species (ROS)

Using Multicolor ROS Brite™ Reagents

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen (such as superoxide, hydroxyl radical, singlet oxygen and peroxides). ROS is highly reactive due to the presence of unpaired valence shell electrons. ROS forms as a natural byproduct of the normal metabolism of oxygen and plays important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. It may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. ROS is also generated by exogenous sources such as ionizing radiation. Under the conditions of oxidative stress, greatly increased production of ROS results in subsequent alteration of membrane lipids, proteins and nucleic acids. Oxidative damage of these biomolecules is associated with aging as well as with a variety of pathological events, including atherosclerosis, carcinogenesis, ischemic reperfusion injury, and neurodegenerative disorders.

ROS Brite™ reagents are a series of new fluorogenic probes to measure oxidative stress in cells. The cell-permeant ROS Brite™ reagents are nonfluorescent and produce bright fluorescence upon ROS oxidation. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. ROS Brite™ 570, 670 and 700 reagents have good selectivity to both hydroxyl radical and superoxide.

ROS Brite™ 700 reagent is a new fluorogenic probe to measure oxidative stress in cells using conventional fluorescence microscopy, high-content imaging, microplate fluorometry, or flow cytometry. The cell-permeant ROS Brite™ 570 reagent is nonfluorescent and produces bright orange fluorescence upon ROS oxidation.

ROS Brite™ 670 can be well excited with He-Ne laser at 633 nm, produces bright orange fluorescence upon ROS oxidation. The resulting fluorescence can be measured using a fluorescence microscope using FITC channel.

ROS Brite™ 700 in PBS buffer (pH 7.2).

Figure 2.5. The fluorescence spectra of ROS Brite™ 570, ROS Brite™ 670 and ROS Brite™ 700 in PBS buffer (pH 7.2).

Figure 2.6. The responses of ROS Brite™ 570 to different ROS species.

Table 2.3. Product Ordering Information

<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>16000</td>
<td>ROS Brite™ 570</td>
<td>1 mg</td>
<td>536</td>
<td>566</td>
</tr>
<tr>
<td>16002</td>
<td>ROS Brite™ 670</td>
<td>1 mg</td>
<td>650</td>
<td>666</td>
</tr>
<tr>
<td>16004</td>
<td>ROS Brite™ 700</td>
<td>1 mg</td>
<td>680</td>
<td>706</td>
</tr>
<tr>
<td>16045</td>
<td>ROS Brite™ APF</td>
<td>1 mg</td>
<td>492</td>
<td>515</td>
</tr>
<tr>
<td>16051</td>
<td>ROS Brite™ HFP</td>
<td>1 mg</td>
<td>492</td>
<td>515</td>
</tr>
</tbody>
</table>
Recent Citations of Fluorescence Indicators


iFluor™ 594, a New Bright Fluorescent Labeling Dye
an Excellent Replacement to Texas Red®, DyLight™ 594 and Alexa Fluor® 594 Dyes

Quick Summary

<table>
<thead>
<tr>
<th>Ex (nm)</th>
<th>Em (nm)</th>
<th>EC (cm^3/M^6)</th>
<th>CF&lt;sub&gt;260 nm&lt;/sub&gt;</th>
<th>CF&lt;sub&gt;280 nm&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>586</td>
<td>611</td>
<td>120,000</td>
<td>0.0605</td>
<td>0.0945</td>
</tr>
</tbody>
</table>

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

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 auto-fluorescence 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.

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

Table 4.1. Typical acceptors for the time-resolved luminescent probes

<table>
<thead>
<tr>
<th>trFluor™ Donors</th>
<th>Recommended Acceptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>trFluor™ Eu</td>
<td>iFluor™ 647, TFS, APC</td>
</tr>
<tr>
<td>trFluor™ Tb</td>
<td>iFluor™ 488, FITC, TF2</td>
</tr>
</tbody>
</table>

Figure 4.1. TR-FRET assay principle using trFluor™ Eu as the donor while Tide Fluor™ 5 (TFS) as the acceptor
trFluor™ Eu TRF Labeling Dye

<table>
<thead>
<tr>
<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>346</td>
<td>617</td>
</tr>
</tbody>
</table>

trFluor™ Tb TRF Labeling Dye

<table>
<thead>
<tr>
<th>Ex (nm)</th>
<th>Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>360</td>
<td>544</td>
</tr>
</tbody>
</table>

**Quick Summary**

- **trFluor™ Tb TRF Labeling Dye**
  - Ex: 360 nm
  - Em: 544 nm

- **trFluor™ Eu TRF Labeling Dye**
  - Ex: 346 nm
  - Em: 617 nm

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

**Key Features of ReadiLink™ Kits:**
- Complete, all the components provided in the kits.
- Robust, only two simple mixing steps required.
- Rapid, less than 10 minutes hands-on time.

**ReadiLink™ iFluor™ Antibody Labeling Kits**

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

**Table 4.2. Product Ordering Information**

<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>1300</td>
<td>ReadiLink™ trFluor™ Eu Protein Labeling Kit</td>
<td>2 labelings</td>
<td>346</td>
<td>617</td>
</tr>
<tr>
<td>1305</td>
<td>ReadiLink™ trFluor™ Tb Protein Labeling Kit</td>
<td>2 labelings</td>
<td>330</td>
<td>544</td>
</tr>
<tr>
<td>16518</td>
<td>trFluor™ Eu Goat Anti-Mouse IgG (H+L)</td>
<td>100 μg</td>
<td>346</td>
<td>617</td>
</tr>
<tr>
<td>16668</td>
<td>trFluor™ Eu Goat Anti-Rabbit IgG (H+L)</td>
<td>100 μg</td>
<td>346</td>
<td>617</td>
</tr>
<tr>
<td>1434</td>
<td>trFluor™ Eu Maleimide</td>
<td>100 μg</td>
<td>346</td>
<td>617</td>
</tr>
<tr>
<td>16925</td>
<td>trFluor™ Eu-Streptavidin Conjugate</td>
<td>100 μg</td>
<td>346</td>
<td>617</td>
</tr>
<tr>
<td>1433</td>
<td>trFluor™ Eu Succinimidyl Ester</td>
<td>1 mg</td>
<td>346</td>
<td>617</td>
</tr>
<tr>
<td>16519</td>
<td>trFluor™ Tb Goat Anti-Mouse IgG (H+L)</td>
<td>100 μg</td>
<td>330</td>
<td>544</td>
</tr>
<tr>
<td>16669</td>
<td>trFluor™ Tb Goat Anti-Rabbit IgG (H+L)</td>
<td>100 μg</td>
<td>330</td>
<td>544</td>
</tr>
<tr>
<td>1444</td>
<td>trFluor™ Tb Maleimide</td>
<td>100 μg</td>
<td>330</td>
<td>544</td>
</tr>
<tr>
<td>16926</td>
<td>trFluor™ Tb-Streptavidin Conjugate</td>
<td>100 μg</td>
<td>330</td>
<td>544</td>
</tr>
<tr>
<td>1443</td>
<td>trFluor™ Tb Succinimidyl Ester</td>
<td>1 mg</td>
<td>330</td>
<td>544</td>
</tr>
</tbody>
</table>

**Table 4.3. Product Ordering Information**

<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>1220</td>
<td>ReadiLink™ iFluor™ 350 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>345</td>
<td>442</td>
</tr>
<tr>
<td>1255</td>
<td>ReadiLink™ iFluor™ 488 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>491</td>
<td>514</td>
</tr>
<tr>
<td>1227</td>
<td>ReadiLink™ iFluor™ 555 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>559</td>
<td>569</td>
</tr>
<tr>
<td>1230</td>
<td>ReadiLink™ iFluor™ 594 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>592</td>
<td>614</td>
</tr>
<tr>
<td>1260</td>
<td>ReadiLink™ iFluor™ 633 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>638</td>
<td>655</td>
</tr>
<tr>
<td>1235</td>
<td>ReadiLink™ iFluor™ 647 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>654</td>
<td>674</td>
</tr>
<tr>
<td>1240</td>
<td>ReadiLink™ iFluor™ 660 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>682</td>
<td>701</td>
</tr>
<tr>
<td>1245</td>
<td>ReadiLink™ iFluor™ 700 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>693</td>
<td>712</td>
</tr>
<tr>
<td>1250</td>
<td>ReadiLink™ iFluor™ 750 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>753</td>
<td>779</td>
</tr>
<tr>
<td>1265</td>
<td>ReadiLink™ iFluor™ 790 Antibody Labeling Kit</td>
<td>2 labelings</td>
<td>782</td>
<td>811</td>
</tr>
</tbody>
</table>
Austria:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Australia:
Life Research Pty Ltd.
Email: info@liferesearch.com
Website: http://www.liferesearch.com

Belgium:
Gentaur BVBA
Email: info@gentaur.com
Website: http://www.gentaur.com

Canada:
Cedarlane Laboratories Ltd.
Email: sales@cedarlanelabs.com
Website: http://www.cedarlanelabs.com

China:
AmyJet Scientific Inc.
Email: amyjetsci@gmail.com
Website: http://www.amyjet.com
Beijing Zhonghao Shidai Co., Ltd
Email: info@biopcr.com
Website: http://www.biopcr.com
Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com
Tianjin Biolite Biotech Co., Ltd
Email: info@tjbiolite.com
Website: http://www.tjbiolite.com

Croatia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Czech Republic:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

Denmark:
Nordic BioSite ApS
Email: info@nordicbiosite.dk
Website: http://www.nordicbiosite.dk

Estonia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Finland:
Nordic BioSite OY
Email: info@biosite.fi
Website: http://www.biosite.fi

France:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Germany:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Hong Kong:
Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com

Hungary:
IZINTA Trading Co., Ltd.
Email: baloghk@izinta.hu
Website: http://www.izinta.hu

Iceland:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

India:
Biochem Life Sciences
Email: info@bcls.in
Website: http://www.bcls.in
GenxBio Health Sciences Pvt. Ltd,
Email: genxbio@gmail.com
Website: http://www.genxbio.com

Ireland:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

Israel:
ADVANSYS Technologies for Life Ltd.
Email: info@advansys.co.il
Website: http://www.advansys.co.il

Italy:
Space Import Export S.r.l
Email: info@spacearl.com
Website: http://www.spacearl.com
Valter Occhiena S.r.l
Email: vo@valterocchiena.com
Website: http://www.valterocchiena.com

Japan:
Cosmo Bio Co., Ltd,
Email: mail@cosmobio.co.jp
Website: http://www.cosmobio.co.jp
Nacalai Tesque, Inc.
Email: info@nacalaiusa.com
Website: http://www.nacalai.com
Wako Pure Chemical Industries, Ltd.
Email: labchem-tec@wako-chem.co.jp
Website: http://www.wako-chem.co.jp

Korea:
Cheong Myung Science Corporation
Email: cms@cmsgcorp.co.kr
Website: http://www.cmsgcorp.co.kr

Latvia and Lithuania:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Netherlands:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Norway:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Poland and Slovenia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Romania:
SC VitroBioChem SRL
Email: office@vitro.ro
Website: http://www.vitro.ro

Singapore and Other South Asian Countries:
BST Scientific Pte Ltd.
Email: info@bstsci.com
Website: http://www.bstsci.com

Slovakia:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

South American Countries and Regions:
Impex Comércio Internacional Ltda.
Email: impexcom@terra.com.br
Website: http://www.impexbrasil.com.br

Spain and Portugal:
Deltacon S. L
Email: info@deltacon.com
Website: http://www.deltacon.com

Sweden:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Switzerland:
LuBioScience GmbH
Email: info@lubio.ch
Website: http://www.lubio.ch

Taiwan:
Rainbow Biotechnology Co., LTD.
Email: rainbow@rainbowbiotech.com.tw
Website: http://www.rainbowbiotech.com.tw

Turkey:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

United Kingdom:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

International Distributors

Interchim
Email: interchim@interchim.com
Website: http://www.interchim.com

Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

LuBioScience GmbH
Email: info@lubio.ch
Website: http://www.lubio.ch

Rainbow Biotechnology Co., LTD.
Email: rainbow@rainbowbiotech.com.tw
Website: http://www.rainbowbiotech.com.tw

Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Austria:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Australia:
Life Research Pty Ltd.
Email: info@liferesearch.com
Website: http://www.liferesearch.com

Belgium:
Gentaur BVBA
Email: info@gentaur.com
Website: http://www.gentaur.com

Canada:
Cedarlane Laboratories Ltd.
Email: sales@cedarlanelabs.com
Website: http://www.cedarlanelabs.com

China:
AmyJet Scientific Inc.
Email: amyjetsci@gmail.com
Website: http://www.amyjet.com
Beijing Zhonghao Shidai Co., Ltd
Email: info@biopcr.com
Website: http://www.biopcr.com
Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com
Tianjin Biolite Biotech Co., Ltd
Email: info@tjbiolite.com
Website: http://www.tjbiolite.com

Croatia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Czech Republic:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

Denmark:
Nordic BioSite ApS
Email: info@nordicbiosite.dk
Website: http://www.nordicbiosite.dk

Estonia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Finland:
Nordic BioSite OY
Email: info@biosite.fi
Website: http://www.biosite.fi

France:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Germany:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Hong Kong:
Mai Bio Co., Ltd
Email: info@maibio.com
Website: http://www.maibio.com

Hungary:
IZINTA Trading Co., Ltd.
Email: baloghk@izinta.hu
Website: http://www.izinta.hu

Iceland:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

India:
Biochem Life Sciences
Email: info@bcls.in
Website: http://www.bcls.in
GenxBio Health Sciences Pvt. Ltd,
Email: genxbio@gmail.com
Website: http://www.genxbio.com

Ireland:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

Israel:
ADVANSYS Technologies for Life Ltd.
Email: info@advansys.co.il
Website: http://www.advansys.co.il

Italy:
Space Import Export S.r.l
Email: info@spacearl.com
Website: http://www.spacearl.com
Valter Occhiena S.r.l
Email: vo@valterocchiena.com
Website: http://www.valterocchiena.com

Japan:
Cosmo Bio Co., Ltd,
Email: mail@cosmobio.co.jp
Website: http://www.cosmobio.co.jp
Nacalai Tesque, Inc.
Email: info@nacalaiusa.com
Website: http://www.nacalai.com
Wako Pure Chemical Industries, Ltd.
Email: labchem-tec@wako-chem.co.jp
Website: http://www.wako-chem.co.jp

Korea:
Cheong Myung Science Corporation
Email: cms@cmsgcorp.co.kr
Website: http://www.cmsgcorp.co.kr

Latvia and Lithuania:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Netherlands:
EUROMEDEX
Email: research@euromedex.com
Website: http://www.euromedex.com

Norway:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Poland and Slovenia:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

Romania:
SC VitroBioChem SRL
Email: office@vitro.ro
Website: http://www.vitro.ro

Singapore and Other South Asian Countries:
BST Scientific Pte Ltd.
Email: info@bstsci.com
Website: http://www.bstsci.com

Slovakia:
Scintila, s.r.o.
Email: rejtharkova@scintila.cz
Website: http://www.scintila.cz

South American Countries and Regions:
Impex Comércio Internacional Ltda.
Email: impexcom@terra.com.br
Website: http://www.impexbrasil.com.br

Spain and Portugal:
Deltacon S. L
Email: info@deltacon.com
Website: http://www.deltacon.com

Sweden:
Nordic BioSite AB
Email: info@biosite.se
Website: http://www.biosite.se

Switzerland:
LuBioScience GmbH
Email: info@lubio.ch
Website: http://www.lubio.ch

Taiwan:
Rainbow Biotechnology Co., LTD.
Email: rainbow@rainbowbiotech.com.tw
Website: http://www.rainbowbiotech.com.tw

Turkey:
Biomol GmbH
Email: info@biomol.de
Website: http://www.biomol.de

United Kingdom:
Stratech Scientific Ltd.
Email: info@stratech.co.uk
Website: http://www.stratech.co.uk

AAT Bioquest®
Advancing Assay & Test Technologies