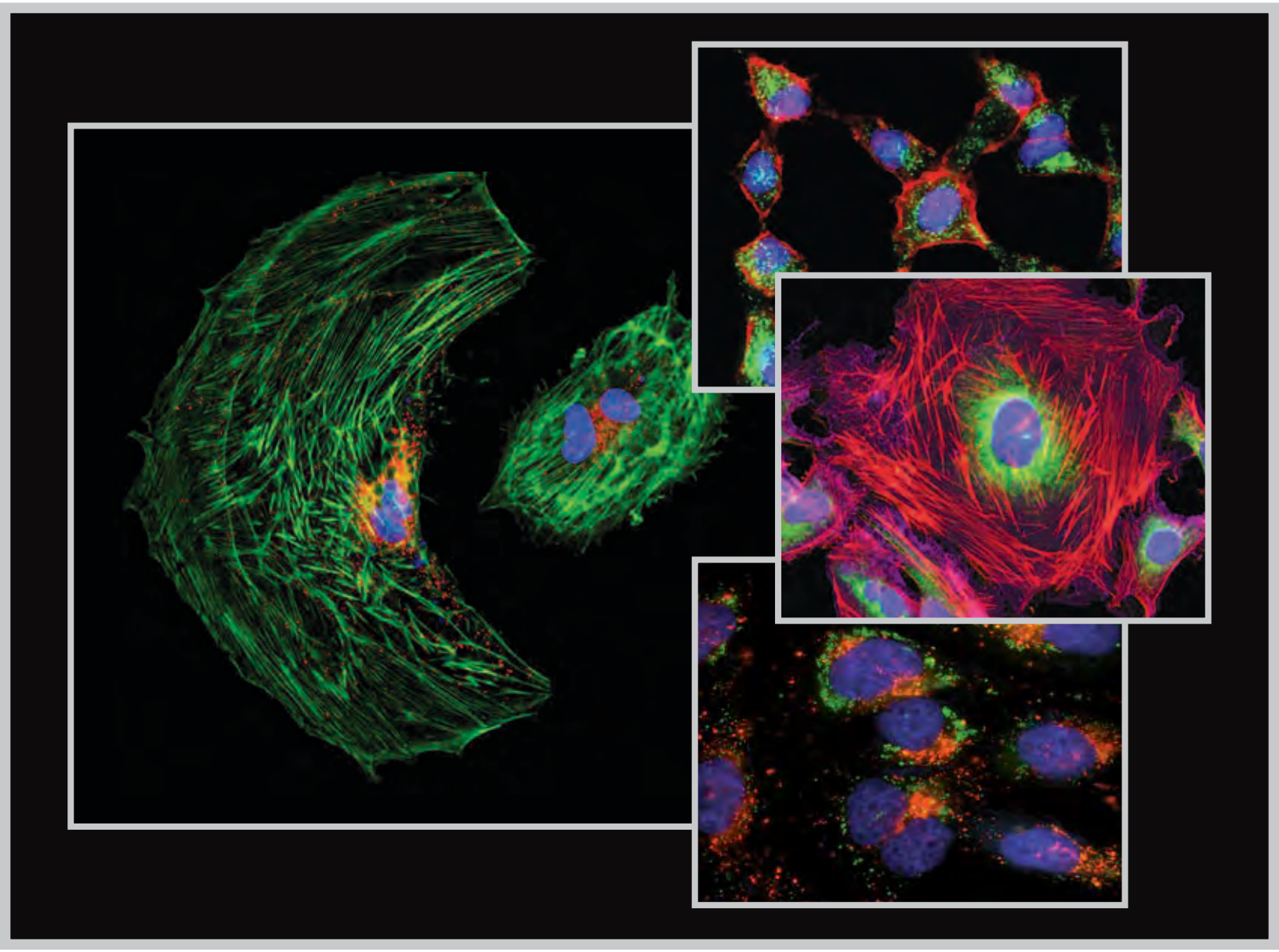
Fluorescence Cell Imaging Probes & Kits



Fluorescence Microscopy | Fluorescence Imaging | High Content Analysis

AAT Bioquest®

Our Mission

AAT Bioquest® is committed to constantly meet or exceed its customer's requirements by providing consistently high quality products and services, and by encouraging continuous improvements in its long-term and daily operations. Our core value is Innovation and Customer Satisfaction.

Our Story

AAT Bioquest®, Inc. develops, manufactures and markets bioanalytical research reagents and kits to life sciences research, diagnostic R&D and drug discovery. We specialize in photometric detections including absorption (color), fluorescence and luminescence technologies. The Company's superior products enable life science researchers to better understand biochemistry, immunology, cell biology and molecular biology. AAT Bioquest offers a rapidly expanding list of enabling products. Besides the standard catalog products, we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays and custom high throughput screening of drug discovery targets.

It is my greatest pleasure to welcome you to AAT Bioquest. We greatly appreciate the constant support of our valuable customers. While we continue to rapidly expand, our core value remains the same: Innovation and Customer Satisfaction. We are committed to being the leading provider of novel biological detection solutions. We promise 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 feedbacks and suggestions from you so that we can better serve your projects.

Very truly yours,

Zhenjun Diwu, Ph.D.
President

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CUSTOMER SERVICE & ORDERING INFORMATION

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2. Delivery: In most cases, we use standard overnight or two-day Federal Express delivery (or equivalent). All shipping charges billed are the responsibility of the customer and are normally prepaid by AAT Bioquest, Inc. and added to the invoice. We reserve the right to make delivery in installments, all such installments to be separately invoiced and paid for when due per invoice, without regard to subsequent deliveries. Partial shipments of available items are made when another item is backordered. Please inspect your packages upon receipt. If the goods have been damaged in transit, we can assist you in filing a claim with the carrier. You shall notify us in writing of any claims for shortages, defects or damages and shall hold the goods for our written instructions concerning disposition. Any claims for such errors must be made within 10 business days. If it is our error, we will do whatever is necessary to ship the correct products as soon as possible. If you shall fail to notify us any defects within 10 days after the goods have been received, such goods shall conclusively be deemed to conform to the terms and conditions and to have been irrevocably accepted by the buyer.

3. Payment: Terms of sale are net 30 days of date of invoice that is sent to you within 24 hours of shipping the order. The amount received must be sufficient to cover both the invoiced amount and any bank charges that may be incurred. Late charges may be added to invoices not paid within the 30-day time period. Late charges must be paid before subsequent orders can be shipped.

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Mitochondrial Labeling Probes and Kits

Mitochondria are membrane-enclosed organelles found in eukaryotic cells that vary considerably in size and structure. Compared to other cellular organelles, mitochondria are unique in the sense that they possess their own mitochondrial DNA (mtDNA) separate from the DNA found in the cell's nucleus. Their composition consists of outer and inner membranes made up of phospholipid bilayers as wells as proteins. This double-membrane organization compartmentalizes mitochondria into five distinct parts each with their own specialized function. One notable function of mitochondria is energy production through oxidative phosphorylation and lipid oxidation. This process involves converting oxygen and nutrients into adenosine triphosphate (ATP). In addition, mitochondria also participate in many other metabolic functions such as signaling, calcium buffering, cellular differentiation or cell death.

Given mitochondria's morphology and functionality they have been linked to or implicated in several human diseases. Mitochondrial morphology is highly variable and controlled by a set of proteins responsible for fission and fusion of the organelle's mass. Mutations to these proteins have been associated with neurological disorders such as Alzheimer disease and Parkinson disease, as well as several other human diseases. With the help of mitochondrion-selective dyes, researchers can evaluate the components involved in mitochondria's morphology and functionality. This provides end-users with a powerful technique to better understand the mechanisms of healthy mitochondria as well as mutated mitochondria that give rise to such diseases.

AAT Bioquest offers a selection of MitoLite™ fluorogenic probes to monitor mitochondrial functionality and morphology. These mitochondrion-selective probes accumulate within the mitochondria via uptake by the mitochondrial membrane potential gradient. They are well-suited for various applications such as investigating the localization and abundance of mitochondria within a cell or mitochondrial activity. In drug discovery, mitochondrial probes aid researchers in observing the effects of pharmaceuticals on numerous pathways that modify mitochondrial functionality. These pathways may include mitochondrial biogenesis, dynamic and mitophagy which maintain or repair proper mitochondrial function.

MitoLite™: Mitochondrion-Selective Probes

AAT Bioquest's MitoLite™ reagents are a set of cationic fluorogenic probes for labeling mitochondria of live cells. These proprietary mitochondrial dyes utilize the mitochondrial membrane potential (MMP) gradient to selectively accumulate in mitochondria. MitoLite™ indicators' hydrophobicity allows them to easily permeate intact live cells. Once inside the mitochondria, MitoLite™ indicators exhibit excellent organelle retention attributed by the cell-retaining groups they carry. This key feature significantly increases the staining efficiency, making them useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity.

MitoLite™ mitochondrion-selective probes are available in a variety of distinct fluorescent colors including blue, green, orange and NIR fluorescence. They have absorption spectra that closely match principal output wavelengths of common excitation sources. MitoLite™ reagents are also well-suited for various applications such as fluorescence microscopy or flow cytometry.

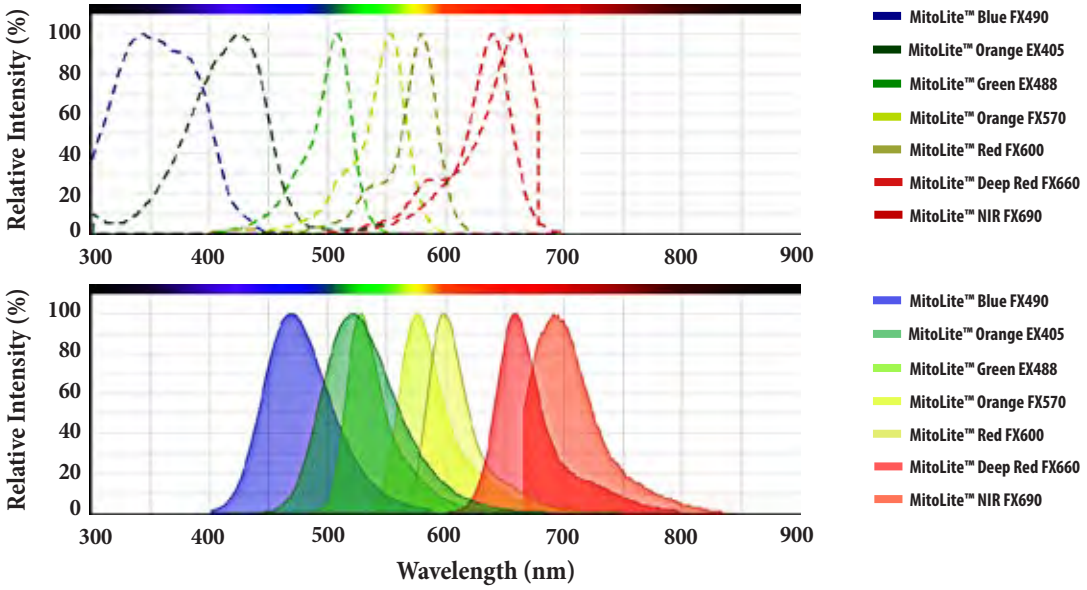

Figure 1.1 Top excitation spectra of MitoLite™ Dyes. Bottom emission spectra of MitoLite™ Dyes.

Cell Navigator™ Mitochondrion Staining Kits

AAT Bioquest's Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as mitochondria, membranes, lysosomes, and nuclei. The selective labeling of live cell compartments provides a powerful method for monitoring cellular events in a spatial and temporal context. Cell Navigator™ mitochondrion staining kits are designed and optimized to label mitochondria of live cells with a full set of fluorescence colors from blue to near infrared. These particular kits utilize MitoLite™ reagents, a proprietary dye that selectively accumulates in mitochondria via uptake through the mitochondrial membrane potential gradient. MitoLite™ mitochondrion-selective indicators are hydrophobic compounds that easily permeate intact live cells. They exhibit excellent photostability and organelle-retention in the mitochondria due to the cell-retaining groups they carry. This key feature contributes to a significant increase in MitoLite™'s staining efficiency.

Cell Navigator™ kits include all essential components with an optimized and robust labeling protocol, requiring minimal hands-on time. They can be readily adapted for various fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. They are effective in a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. Cell Navigator™ kits are suitable for proliferating and non-proliferating cells, as well as for adherent cells or cells in suspension.

In addition to our robust Cell Navigator™ Mitochondrion Staining Kits, we also offer a selection of common mitochondrial stains and probes such as JC-1, TMRE, TMRM and rhodamine 123. Since cyanine and rhodamine mitochondrial stains are positively charged, they are selectively accumulated in the mitochondria via the mitochondrial membrane potential.

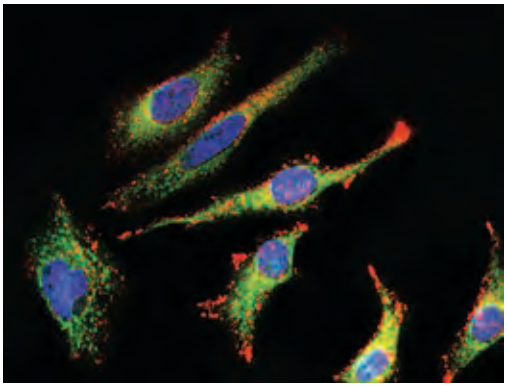

Figure 1.2 Fluorescence image of HeLa cells stained with MitoLite™ Green FM (Green, Cat#22695) using fluorescence microscope with a FITC filter set. Live cells were co-stained with lysosome dye LysoBrite™ Red (Red, Cat#22645) and nuclei stain Nuclear Violet™ LCS1 (Blue, Cat#17543).

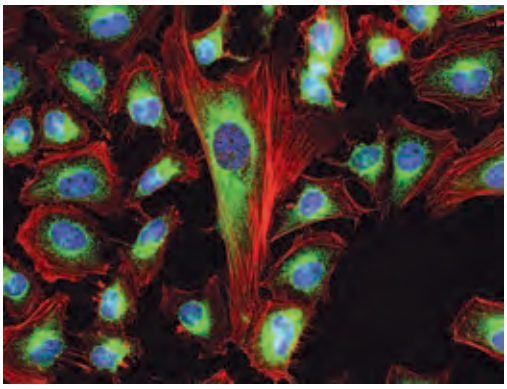

Figure 1.3 Fluorescence image of HeLa cells. Live cells were stained with mitochondria dye MitoLite™ Green (Green, Cat#22675). After fixation in 4% formaldehyde, the cells were labeled with F-actin dye iFluor™ 633-Phalloidin (Red, Cat# 23125) and counterstained with Nuclear Blue™ DCS1 (Blue, Cat# 17548).

Table 1.1 Spectral characteristics of MitoLite™ Dyes

Cat #	MitoLite™ Dyes	Excitation (nm)	Emission (nm)	Fixable
22674	MitoLite™ Blue FX490	350	490	Yes
22675	MitoLite™ Green EX488	498	520	No
22695	MitoLite™ Green FM	491	513	Yes
22676	MitoLite™ Orange FX570	545	575	Yes
22677	MitoLite™ Red FX600	575	600	Yes
22678	MitoLite™ Deep Red FX660	640	659	Yes
22679	MitoLite™ Orange 405	399	550	No
22690	MitoLite™ NIR FX690	660	692	Yes

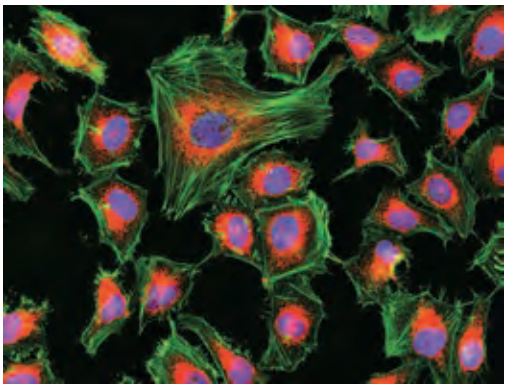

Figure 1.4 Fluorescence image of HeLa cells stained with Cell Navigator™ Mitochondrion Staining Kit *NIR Fluorescence* (Cat#22670) using fluorescence microscope with a Cy5 filter set. Live cells were stained with mitochondria dye MitoLite™ NIR (Red). After fixation, the cells were labeled with F-actin dye iFluor™ 488-Phalloidin (Green, Cat#23115) and counterstained with Nuclear Blue™ DCS1 (Blue, Cat#17548).

Mitochondrial Membrane Potential

Mitochondria contain two major membranes, an outer and inner membrane. The outer mitochondrial membrane contains large numbers of integral membrane proteins known as porins. These porins facilitate the diffusion of ions and small molecules (5 kDa or less) across the outer membrane and into the mitochondria's intermembrane space. The outer membrane also contains various enzymes involved in activities such as the oxidation of epinephrine. Disruption of this membrane contributes to the leakage of these enzymes into the cytosol which is indicative of cell death or apoptosis.

The inner mitochondrial membrane contains proteins responsible for oxidative phosphorylation, ATP synthase for ATP production and proteins necessary for mitochondrial fusion and fission. During oxidative phosphorylation, a series of redox reactions passes electrons along proteins and organic molecules of the electron transport chain in the inner mitochondrial membrane. This series of redox reactions establishes an electrochemical gradient called the mitochondrial membrane potential ($\Delta\psi_M$), which ultimately drives ATP synthesis. With the aid of fluorescent probes, researchers can effectively investigate and monitor these $\Delta\psi_M$ generating reactions to better understand mitochondrial functionality.

Mitochondrial disease or dysfunction is a characteristic of aging and has been linked to various diseases due to the mitochondria's inability to function properly. A reduction in mitochondrial function occurs as a result of adverse changes to the $\Delta\psi_M$ disrupting the mitochondrial membrane. These changes can result in a reduced efficiency of oxidative phosphorylation, a decrease in the production of ATP and the release of apoptogenic factors. In some apoptotic systems, loss of $\Delta\psi_M$ may be an early indicator of the apoptotic process. Because $\Delta\psi_M$ decreases during apoptosis, mitochondrial fluorogenic probes have been developed to selectively target and monitor $\Delta\psi_M$. Such probes include TMRM, TMRE, Rhodamine 123, JC-1 and JC-10™.

JC-1 and JC-10™

JC-1 is a green-fluorescent probe commonly used for monitoring $\Delta\psi_M$ with flow cytometry. It is capable of selectively entering mitochondria, and reversibly changes its color from green to orange as $\Delta\psi_M$ increases (80-100 mV). At low concentrations or low membrane potentials, JC-1 exists as a monomer yielding green fluorescence. However, at higher concentrations or higher membrane potentials, JC-1 forms red-fluorescent "J-aggregates". These aggregates exhibit a broad excitation spectrum and a very narrow emission spectrum. Because J-aggregate formation increases linearly with applied membrane potential over the range of 30–180 mV, this phenomenon can be exploited for potentiometric measurements. JC-1 is more specific for mitochondrial versus plasma membrane potential and more consistent in its response to depolarization than some other cationic dyes such as DiOC6 (3) and rhodamine 123.

Various types of ratio measurements are possible by combining signals of the green-fluorescent JC-1 monomer (Ex/Em = ~514/529 nm) and the red-fluorescent J-aggregate (Ex/Em = ~585/590 nm),

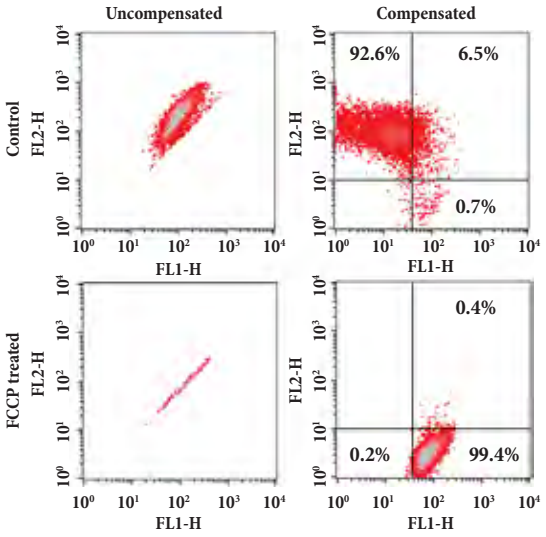

Figure 1.5 Effect of FCCP induced $\Delta\psi_M$ change in Jurkat cells. Jurkat cells were dye loaded with JC-10™ dye loading solution along with DMSO (Top) or 5 µM FCCP (Bottom) for 10 minutes. The fluorescent intensities for both J-aggregates and monomeric forms of JC-10™ were measured with a FACSCalibur (Becton Dickinson) flow cytometer using FL1 and FL2 channels. Uncompensated data (left column) were compared with compensated data (right column).

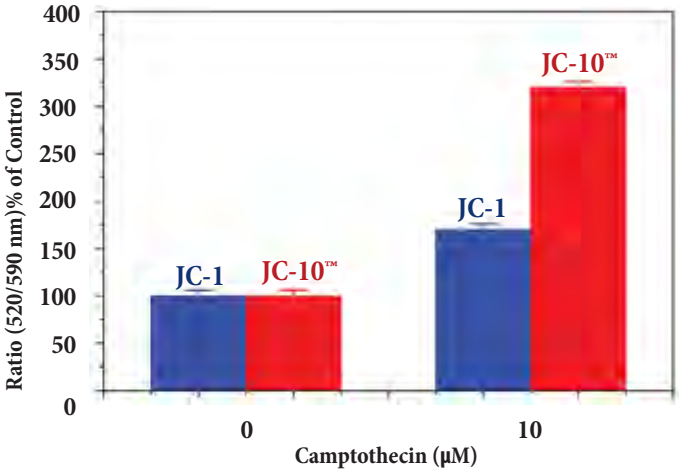

Figure 1.6 Campotothecin-induced $\Delta\psi_M$ changes were measured with JC-10™ and JC-1 in Jurkat cells. After Jurkat cells were treated with camptothecin (10 µM) for 4 hours, JC-1 and JC-10™ dye loading solutions were added to the wells and incubated for 30 minutes. The fluorescent intensities for both J-aggregates and monomeric forms of JC-1 and JC-10™ were measured at Ex/Em = 490/525 nm and 490/590 nm with NOVOstar microplate reader (BMG Labtech).

which can be effectively excited anywhere between 485 nm and its absorption maximum. JC-1 is widely used for detecting mitochondrial depolarization in apoptotic cells and for assaying multidrug-resistant cells. It is also frequently employed for mitochondrial function assessment in cell-based high-throughput assays.

AAT Bioquest has developed JC-10™ as superior alternative to JC-1. JC-10™ has potential-dependent spectroscopic properties similar to those of JC-1 for detecting mitochondrial depolarization in apoptotic cells. JC-10™ is superior and more convenient to use than JC-1 due to its higher sensitivity and improved water solubility. The poor water solubility of JC-1 makes it hard to use for some applications. Even at 1 μM concentration, JC-1 tends to precipitate in aqueous buffer. When high dye concentration is desired, JC-10™ is capable of selectively entering into mitochondria, and changes reversibly its color from green to orange as membrane potentials increase. This property is due to the reversible formation of JC-10™ aggregates upon membrane polarization that causes shifts in emitted light from 520 nm (i.e., emission of JC-10™ monomeric form) to 570 nm (i.e., emission of J-aggregate). When excited at 490 nm, the color of JC-10™ changes reversibly from green to orange as the mitochondrial membrane becomes more polarized. Both colors can be detected using the filters commonly mounted in all flow cytometers. Green emission can be analyzed using fluorescence channel 1 (FL1) and orange emission using channel 2 (FL2). Besides its use in flow cytometry, JC-10™ can also be used in fluorescence imaging.

JC-10™ can be used to investigate and monitor $\Delta\psi_M$ in a wide array of cell types such as neurons and myocytes. JC-10™ probes improved solubility in aqueous solutions and streamlined protocol makes it easier to prepare with minimal hands on-time ensuring consistent results across multiple runs. JC-10™ probes exhibit a better signal intensity compared to other probes and has a significantly higher signal-to-background ratio. It can be used to analyze critical cell processes such as ATP synthesis, cytotoxicity, oxidative stress or apoptosis. Additionally, JC-10™ is a key component in Cell Meter™ JC-10™ Mitochondrial Membrane Potential Assay Kits. This kit has been optimized for flow cytometry as well as for microplate-based high throughput screening applications to screen for both apoptosis activators and inhibitors.

Cell Meter™ Mitochondrion Membrane Potential Assay Kits

Cell Meter™ Mitochondrion Membrane Potential Assay Kits provide all the essential components with an optimized assay protocol well-suited for flow cytometry and microplate assay. These fluorimetric assays use one of our proprietary cationic mitochondrial probes, JC-10™, MitoTell™ Orange or MitoLite™ NIR for detecting $\Delta\psi_M$ changes.

Cell Meter™ Mitochondrial Membrane Potential Assay Kits use our proprietary cationic MitoTell™ Orange dye for the detection of apoptosis in cells by monitoring changes in $\Delta\psi_M$. In normal cells, the orange fluorescence intensity of MitoTell™ Orange increases as it accumulates in the mitochondria. In apoptotic cells, MitoTell™ Orange exhibits a decrease in fluorescence intensity following the collapse of the $\Delta\psi_M$. Cells stained with MitoTell™ Orange can be visualized with a flow cytometer at 488 nm excitation

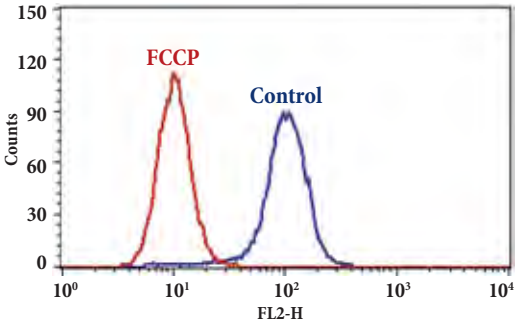

Figure 1.7 The decrease in fluorescence intensity of MitoTell™ Orange with the addition of FCCP in Jurkat cells. Jurkat cells were loaded with MitoTell™ Orange alone (Blue) or in the presence of 30 μM FCCP (Red) for 15 minutes. The fluorescence intensity of MitoTell™ Orange was measured with a FACSCalibur (Becton Dickinson) flow cytometer using FL2 channel.

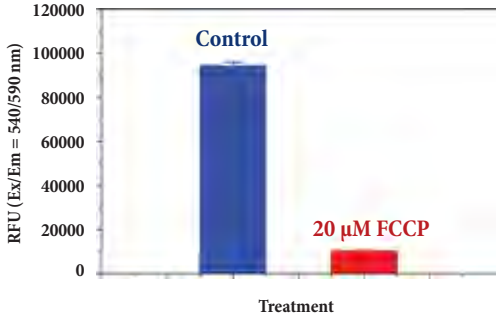

Figure 1.8 The decrease in the fluorescence intensity of MitoTell™ Orange with the addition of FCCP in HeLa cells. HeLa cells were dye loaded with MitoTell™ Orange alone or in the presence of 20 μM FCCP for 15 minutes. The fluorescence intensity of MitoTell™ Orange was measured 30 minutes after adding assay buffer with a FlexStation™ microplate reader (Molecular Devices) at Ex/Em = 540/590 nm (cut off 570 nm, bottom read).

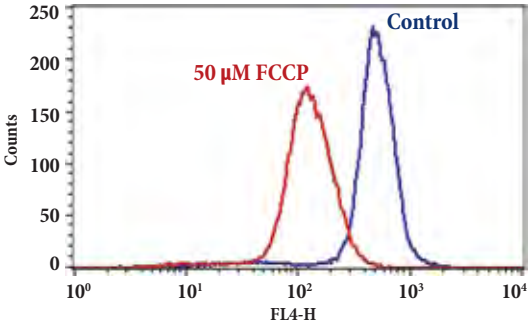

Figure 1.9 The decrease in fluorescence intensity of MitoLite™ NIR with the addition of FCCP in Jurkat cells. Jurkat cells were loaded with MitoLite™ NIR alone (blue) or in the presence of 50 μM FCCP (red) for 10 minutes. The fluorescence intensity of MitoLite™ NIR was measured with a FACSCalibur (Becton Dickinson) flow cytometer using FL4 channel.

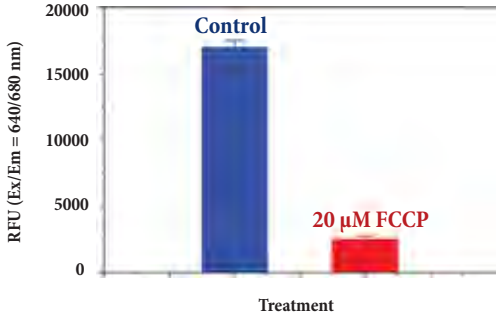

Figure 1.10 The decrease in MitoLite™ NIR fluorescence with the addition of FCCP in HeLa cells. HeLa cells were loaded with MitoLite™ NIR alone or in the presence of 20 μM FCCP for 15 minutes. The fluorescence intensity of MitoLite™ NIR was measured 30 minutes after adding assay buffer with a FlexStation™ microplate reader (Molecular Devices) at Ex/Em = 640/680 nm (cut off 665 nm, bottom read).

with orange emission (FL2 channel). For multi-parametric studies, the orange Cell Meter™ Mitochondrial Membrane Potential Assay Kit optimized for flow cytometry can be used in conjunction with Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22835) to investigate cell vitality and apoptosis. Cell Meter™ Mitochondrial Membrane Potential Assay Kits in orange fluorescence are optimized for screening apoptosis activators and inhibitors with a flow cytometer or a fluorescence microplate reader.

Cell Meter™ NIR Mitochondrial Membrane Potential Assay Kits use our proprietary cationic MitoLite™ NIR for the detection of apoptosis in cells by monitoring changes in $\Delta\psi_M$. In normal cells, the NIR fluorescence intensity of MitoLite™ NIR increases as it accumulates in the mitochondria. In apoptotic cells, MitoLite™ NIR fluorescence intensity decreases following the collapse of $\Delta\psi_M$. Cells stained with MitoLite™ NIR can be visualized with a flow cytometer at red excitation and far red emission (FL4 channel). For multi-parametric studies, the Cell Meter™ NIR Mitochondrial Membrane Potential Assay Kit optimized for flow cytometry can be used together with other reagents, such as propidium iodide and Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22790) for studying cell vitality and apoptosis. Cell Meter™ NIR Mitochondrial Membrane Potential Assay Kits are optimized for screening apoptosis activators and inhibitors with a flow cytometer or a fluorescence microplate reader.

Mitochondrial ROS

Reactive oxygen species (ROS) are chemically reactive species containing oxygen such as hydroxyl radicals, superoxides and peroxides. Under normal conditions, ROS formation is a natural byproduct of oxygen metabolism playing a vital role in cell signaling and homeostasis. However, during environmental stress or oxidative stress, ROS levels drastically increase causing significant damage to cellular organelles such as mitochondria.

Mitochondria, the "energy power-plant" of cells, produce ATP through oxidative phosphorylation. This process transfers protons (H+ ions) across the inner mitochondrial membrane via the electron transport chain through a series of redox reactions. However, under certain metabolic or stress conditions, this can result in ROS overproduction. Increased levels of ROS adversely induce mitochondrial DNA mutations or can alter mitochondrial membrane permeability. Such changes have been implicated in the development of several neurodegenerative diseases such as Alzheimer's or Parkinson's disease.

Hydroxyl Radical Detection

The hydroxyl radical (•OH) is the most reactive oxygen species and has been linked to an assortment of diseases. As a harmful byproduct of oxidative metabolism, •OH radicals cause significant subcellular damage to organelles. Therefore, sensitive and selective detection of intracellular •OH is vital to understanding cellular redox and the impact of its dysregulation on various pathologies. Although a variety of fluorescent sensors exist to detect •OH, their rapid photobleaching, short emission wavelength, and non-selective reactions with other ROS species have limited their applications in cells and tissues. To mitigate this issue, AAT Bioquest has developed MitoROS™ OH580. This novel fluorescent probe selectively targets and detects intracellular •OH in living cells.

Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit (Cat# 16055) is optimized for detecting •OH in mitochondria of live cells with one hour incubation. It includes MitoROS™ OH580, a live-cell permeant probe that can rapidly and selectively target •OH in live cells. In the absence of •OH, the probe exhibits little to no fluorescence. It is not until it reacts with •OH, that it generates a strong red fluorescence that can be easily read at Ex/Em= 540/590 nm. These fluorogenic characteristics contribute to MitoROS™ OH580 high signal-to-background ratio. This kit is well-suited for

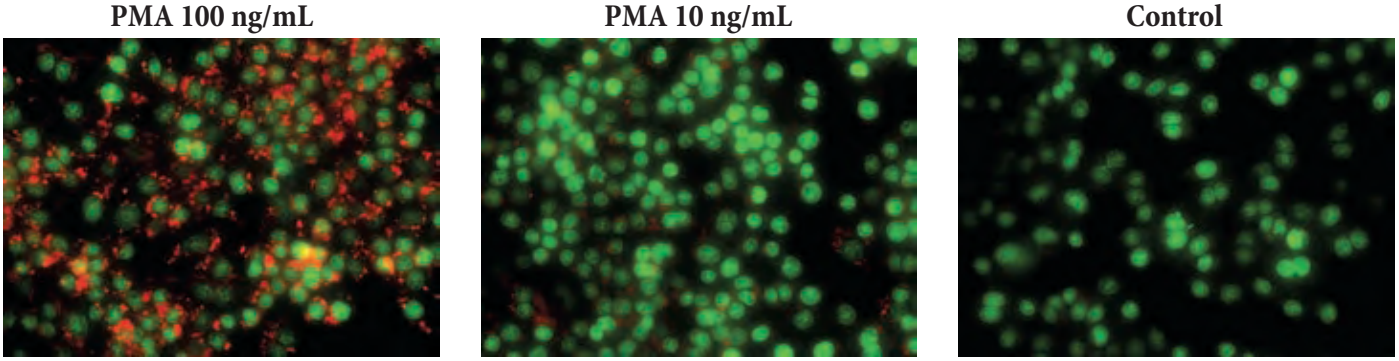

Figure 1.11 Fluorescence images of mitochondrial hydroxyl radical (•OH) in RAW 264.7 macrophage cells. Cells stained with MitoROS OH580 (Red) were treated with PMA (phorbol 12-myristate 13-acetate) in the range of 0-100 ng/mL in growth medium at 37 °C for 4 hours to stimulate endogenous hydroxyl radical. Nuclei were counterstained with Nuclear Green LCS1 (Green, Cat#17540).

fluorescence microplate readers and fluorescence microscopy serving as a valuable tool for life science research as well as medical diagnostic applications.

Superoxide Detection

Hydroethidine, a redox-sensitive probe, has been widely used to detect intracellular superoxide anion (• O_2^-). It is a common assumption that the reaction between superoxide and hydroethidine results in the formation of a two-electron oxidized product, ethidium, which binds to DNA and enhances fluorescence (excitation, 500 - 530 nm; emission, 590 - 620 nm). Hydroethidine operates effectively as a probe for the measurement of reactive oxygen species. The dye enters cells freely and is oxidized to ethidium bromide. The probe has been used extensively with natural killer (NK) cells and as a vital dye for identification of proliferation and hypoxic cells in tumors. Studies have been performed using neutrophils and endothelial cells as well as HL60 cells and macrophages. A major advantage of this probe is its ability to distinguish between superoxide and H_2O_2.

MitoROS™ 580 is a superoxide-sensitive dye that is localized in mitochondria upon loading into live cells. Oxidation of MitoROS™ 580 by superoxide generates red fluorescence. MitoROS™ 580 can be used for monitoring superoxide in mitochondria with either a fluorescence microscope or a fluorescence flow cytometer. MitoROS™ 580 reagent permeates live cells where it selectively targets mitochondria. It is rapidly

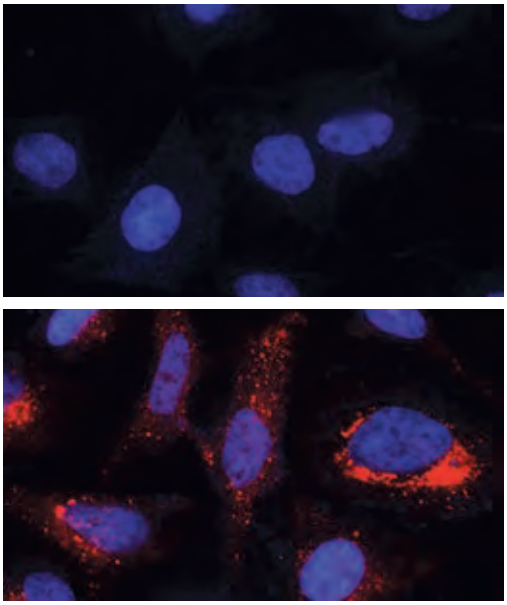

Figure 1.14 Fluorescence images of hydroxyl radical measurement in HeLa cells using Cell Meter™ Mitochondriol Hydroxyl Radical Detection Kit (Cat#16055). Control (Top): HeLa cells were kept in 1X HBSS buffer without treatment. Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17530). Fenton Reaction (Bottom): Cells were then treated with 10 µM $CuCl_2$ and 100 µM H_2O_2 in 1X HBSS buffer at 37 °C for 1 hour. After washing 3 times with HHBS, HeLa cells were measured using a fluorescence microscope with a TRITC filter set (Red).

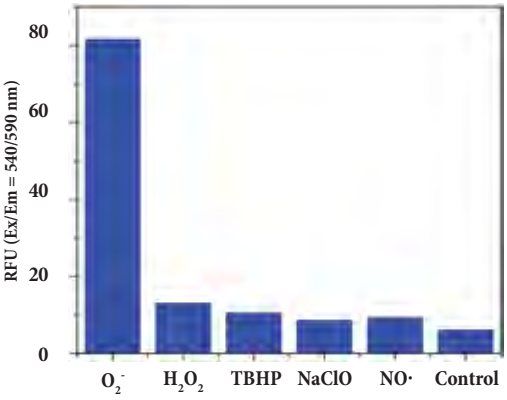

Figure 1.15 Fluorescence response of MitoROS™ 580 (10 µM, Cat#16052)) to different reactive oxygen species (ROS) and reactive nitrogen species (RNS). The fluorescence intensities were monitored at Ex/Em = 540/590 nm.

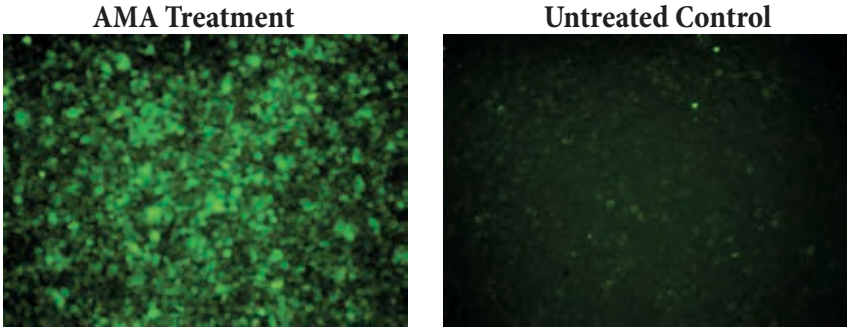

Figure 1.12 Fluorescence images of superoxide measurement in macrophage cells using Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit (Cat#16060). RAW 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. AMA Treatment: Cells were treated with 5 µM Antimycin A (AMA) at 37 °C for 2 hours, then incubated with MitoROS™ 520 for 1 hour. Untreated Control: RAW 264.7 cells were incubated with MitoROS™ 520 at 37 °C for 1 hour without AMA treatment. The fluorescence signal was measured using fluorescence microscope with a FITC filter

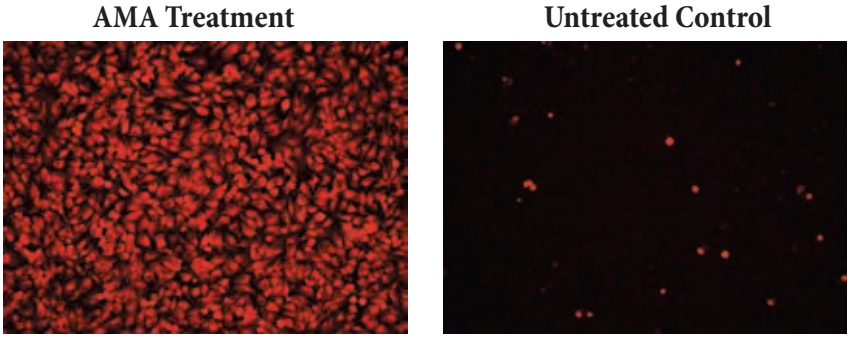

Figure 1.13 Fluorescence images of superoxide measurement in HeLa cells using Cell Meter™ Fluorimetric Intracellular Superoxide Detection Kit (Cat#22971). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. AMA Treatment: Cells were treated with 50 µM Antimycin A (AMA) at 37 °C for 30 minutes, then incubated with MitoROS™ 580 for 1 hour. Untreated Control: HeLa cells were incubated with MitoROS™ 580 (Cat#16052) at 37 °C for 1 hour without AMA treatment. The fluorescence signal was measured using fluorescence microscope with a TRITC filter.

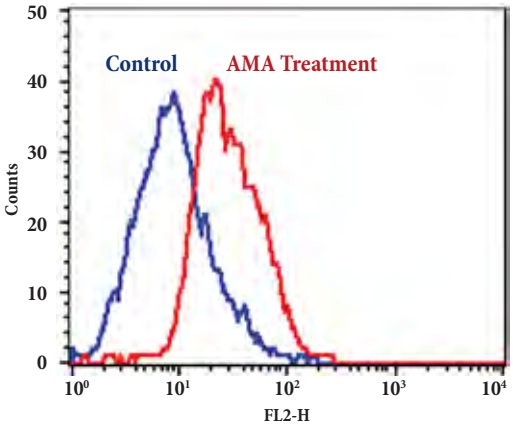

Figure 1.16 Detection of intracellular superoxide in Jurkat cells using Cell Meter™ Fluorimetric Intracellular Superoxide Activity Assay Kit (Cat#22970). AMA Treatment (Red): Cells were treated with 50 µM Antimycin A (AMA) at 37 °C for 30 minutes, then incubated with MitoROS™ 580 for 1 hour. Control (Blue): Cells were incubated with MitoROS™ 580 at 37 °C for 1 hour without AMA treatment. The fluorescence signal was monitored at FL2 channel using a flow cytometer (BD FACSCalibur).

oxidized by superoxide and is less likely to be oxidized by other reactive oxygen species (ROS) and reactive nitrogen species (RNS). The oxidized product is highly fluorescent in cells. MitoROS™ 580 provides a valuable tool for investigating oxidative stress in various pathologies.

The detection of intracellular mitochondrial superoxide is of great importance to understanding proper cellular redox regulation and the impact of its dysregulation on various pathologies. Cell Meter™ Fluorimetric Mitochondrial Superoxide Detection Kits (Cat# 16060, 22970 & 22971) use our unique MitoROS™ superoxide indicators, to quantify superoxide level in live cells. MitoROS™ sensors are cell permeant and can rapidly and selectively detect superoxide in mitochondria. The Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kits provide a sensitive, one-step fluorimetric assay to detect mitochondrial superoxide in live cells with one hour incubation.

Product Ordering Information For Mitochondria Staining Probes and Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
16060	Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit *Green Fluorescence*	200 Tests	509	534
22970	Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit*Optimized for Flow Cytometry*	100 Tests	540	590
22971	Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit*Optimized for Microplate Reader*	200 Tests	540	590
11505	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Blue Fluorescence Optimized for Flow Cytometry*	100 Tests	405	450
11504	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Blue Fluorescence*	100 Tests	405	450
11506	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 Tests	490	530
11503	Cell Meter™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit *Green Fluorescence*	200 Tests	492	515
22801	Cell Meter™ JC-10™ Mitochondrion Membrane Potential Assay Kit *Optimized for Flow Cytometry Assays*	100 Tests	510	525
22800	Cell Meter™ JC-10™ Mitochondrion Membrane Potential Assay Kit *Optimized for Microplate Assays*	500 Tests	510	525
16055	Cell Meter™ Mitochondrial Hydroxyl Radical Detection Kit *Red Fluorescence*	200 Tests	576	598
22804	Cell Meter™ Mitochondrion Membrane Potential Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*	500 Tests	546	575
22805	Cell Meter™ Mitochondrion Membrane Potential Assay Kit *Orange Fluorescence Optimized for Microplate Reader*	500 Tests	546	575
22802	Cell Meter™ NIR Mitochondrion Membrane Potential Assay Kit *Optimized for Flow Cytometry*	500 Tests	646	659
22803	Cell Meter™ NIR Mitochondrion Membrane Potential Assay Kit *Optimized for Microplate Reader*	500 Tests	646	659
22665	Cell Navigator™ Mitochondrion Staining Kit *Blue Fluorescence*	500 Assays	350	490
22669	Cell Navigator™ Mitochondrion Staining Kit *Deep Red Fluorescence*	500 Assays	640	659
22666	Cell Navigator™ Mitochondrion Staining Kit *Green Fluorescence*	500 Assays	498	520
22670	Cell Navigator™ Mitochondrion Staining Kit *NIR Fluorescence*	500 Assays	660	693
22673	Cell Navigator™ Mitochondrion Staining Kit *Orange Fluorescence with 405 nm Excitation*	500 Assays	399	550
22667	Cell Navigator™ Mitochondrion Staining Kit *Orange Fluorescence*	500 Assays	545	575
22668	Cell Navigator™ Mitochondrion Staining Kit *Red Fluorescence*	500 Assays	575	600
22200	JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide] *CAS#: 3520-43-2*	5 mg	515	529
22201	JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide] *CAS#: 3520-43-2*	50 mg	515	529
22204	JC-10™ *Superior alternative to JC-1*	5x100 µL	510	525
22674	MitoLite™ Blue FX490	500 Tests	350	490
22675	MitoLite™ Green EX488	500 Tests	498	520
22695	MitoLite™ Green FM	10x50 µg	491	513
22678	MitoLite™ NIR 660	500 Tests	640	659
22690	MitoLite™ NIR FX690	500 Tests	660	693
22679	MitoLite™ Orange EX405	500 Tests	399	550
22676	MitoLite™ Orange FX570	500 Tests	545	575
22677	MitoLite™ Red FX600	500 Tests	575	600
16052	MitoROS™ 580 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochnodria*	500 Tests	510	580
22210	Rhodamine 123 *CAS 62669-70-9*	25 mg	507	529
22220	TMRE [Tetramethylrhodamine ethyl ester] *CAS#: 115532-52-0*	25 mg	549	574
22221	TMRM [Tetramethylrhodamine methyl ester] *CAS#: 115532-50-8*	25 mg	549	573

Lysosome Labeling Probes and Kits

Table 2.1 Spectral characteristics of LysoBrite™ Dyes

Cat #	LysoBrite™ Dyes	Excitation (nm)	Emission (nm)
22642	LysoBrite™ Blue	353	442
22646	LysoBrite™ Deep Red	596	619
22643	LysoBrite™ Green	450	505
22641	LysoBrite™ NIR	636	650
22644	LysoBrite™ Orange	542	556
22645	LysoBrite™ Red	575	597

Key Features of Cell Navigator™ Lysosome Staining Kits

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

Lysosomes are the "waste-disposal" system of the cell digesting unwanted materials and cellular debris in the cytoplasm. These membrane-enclosed organelles contain hydrolytic enzymes that can hydrolyze proteins, peptides, nucleic acids, carbohydrates and lipids. Lysosomes are capable of fusing with other organelles allowing lysosomal hydrolytic enzymes to digest the organelle's contents. Lysosomal morphology varies in size from 0.1-1.2 µm and maintains an interior pH of 4.8 which is optimal for hydrolytic enzyme activity. The lysosome maintains this acidic pH by pumping protons across the membrane via proton pumps and chloride ion channels. Additionally, the lysosomal membrane protects the rest of the cell from its degradative enzymes to prevent unwanted lysing of cytosolic molecules and organelles.

LysoBrite™: Lysosomal Labeling Probes

Synthesis of lysosomal hydrolytic enzymes is controlled by nuclear genes. Mutations in these genes account for an array of inherited metabolic disorders which results from defects in lysosomal enzyme functionality. These metabolic disorders are collectively known as lysosomal storage diseases. Defects to lysosomal hydrolytic enzymes result in the accumulation of macromolecules or monomeric compounds contributing to abnormal signaling pathways which ultimately lead to pathogenic disorders. To monitor and investigate the biosynthesis and

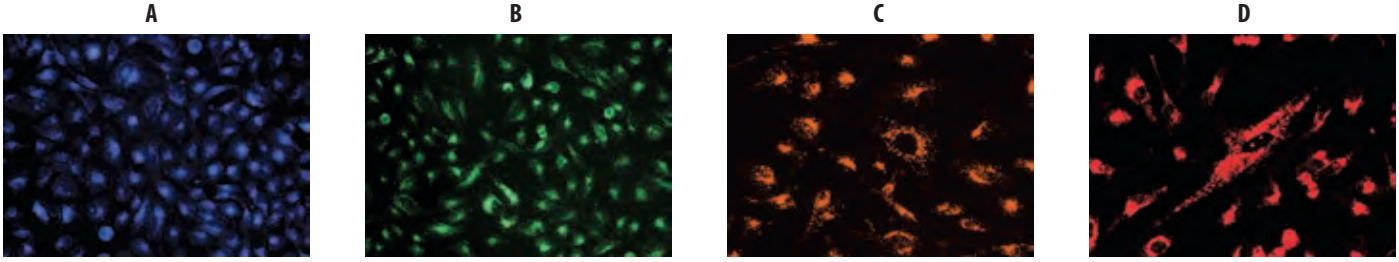

Figure 2.1 Four-panel composite image of HeLa cells stained with Cell Navigator™ Lysosomal Staining Kits in a Costar black wall or clear bottom 96-well plate. **A**) Image of HeLa cells stained with LysoBrite™ Blue (Blue, Cat#22642). **B**) Image of HeLa cells stained with LysoBrite™ Green (Green, Cat#22643). **C**) Image of HeLa cells stained with LysoBrite™ Orange (Orange, Cat#22644). **D**) Image of HeLa cells stained with LysoBrite™ Red (Red, Cat#22645).

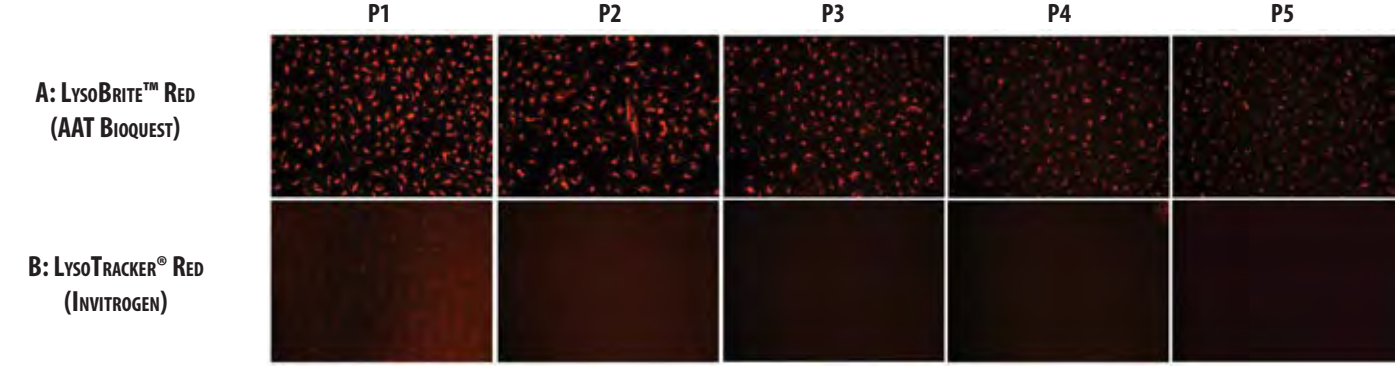

Figure 2.2 Images of HeLa cells stained with **A**: Cell Navigator™ Lysosome Staining Kit (Top, from AAT Bioquest, Cat#22658), **B**: LysoTracker® Red DND-99 (Bottom, from Invitrogen) in a Costar black wall/clear bottom 96-well plate. The signals were compared at 5 cell passages (P1, P2, P3, P4 and P5) respectively using an Olympus fluorescence microscope.

pathogenesis of lysosomes, AAT Bioquest has developed LysoBrite™ probes to selectively target and label lysosomes of live cells.

Our Cell Navigator™ Lysosome Staining Kits utilize our proprietary LysoBrite™ indicators to selectively accumulate in lysosomes of live cells via the lysosome pH gradient. The hydrophobic composition of LysoBrite™ dyes facilitates permeation of intact live cells. Upon entering lysosomes, the fluorescence intensity of LysoBrite™ dyes significantly enhances. This key feature greatly reduces background interference making them advantageous for a variety of studies, including cell adhesion, chemo-taxis, multidrug resistance, cell-viability, apoptosis and cytotoxicity. Cell Navigator™ staining kits are well-suited for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. Kits include robust labeling protocols that minimize end-user hands-on time, and are adapted for many fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope.

Cell Meter™ Autophagy Imaging Kit

Autophagy is a natural and regulated lysosomal degradation pathway essential for cell viability, development and homeostasis. It is the destructive mechanism of cells that degrades and recycles unnecessary or dysfunctional cellular components. It achieves this through the sequestration of targeted cytoplasmic materials into double-membrane bound organelles known as autophagosomes. These autophagosomes later fuse with lysosomal organelles to have their contents digested and recycled by lysosomal enzymes. Autophagy serves as an adaptive mechanism to protect organisms against various pathologies. To investigate the role autophagy plays in cell homeostasis, imaging tools and assays have been developed to monitor autophagy functionality in response to cellular stress,

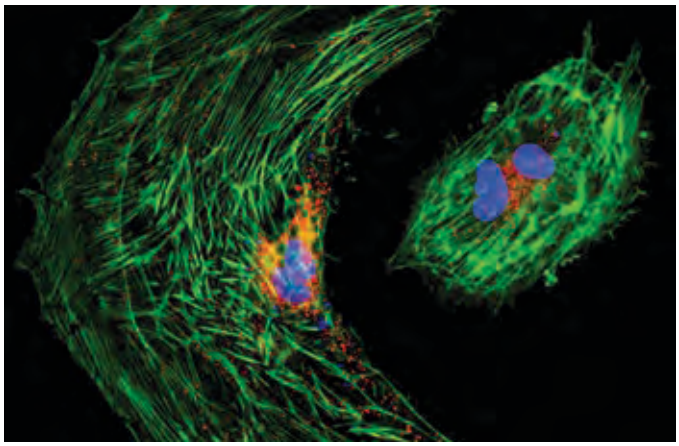

Figure 2.3 Fluorescence images of CPA cells co-stained with lysosome dye LysoBrite™ Red (Red, Cat#22658), Phalloidin-iFluor™ 488 Conjugate (Green, Cat#23115) to label actin filaments, and nuclei stain Nuclear Blue™ DCS1 (Blue, Cat#17548). The cells were fixed in 4% formaldehyde.

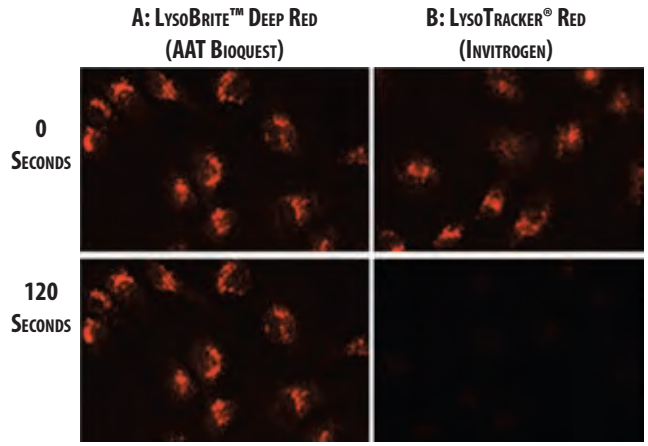

Figure 2.4 Images of HeLa cells stained with **A**: Cell Navigator™ Lysosome Staining Kit (Cat#22659), **B**: LysoTracker® Red DND-99 (from Invitrogen) in a Costar black wall/clear bottom 96-well plate. The TRITC signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

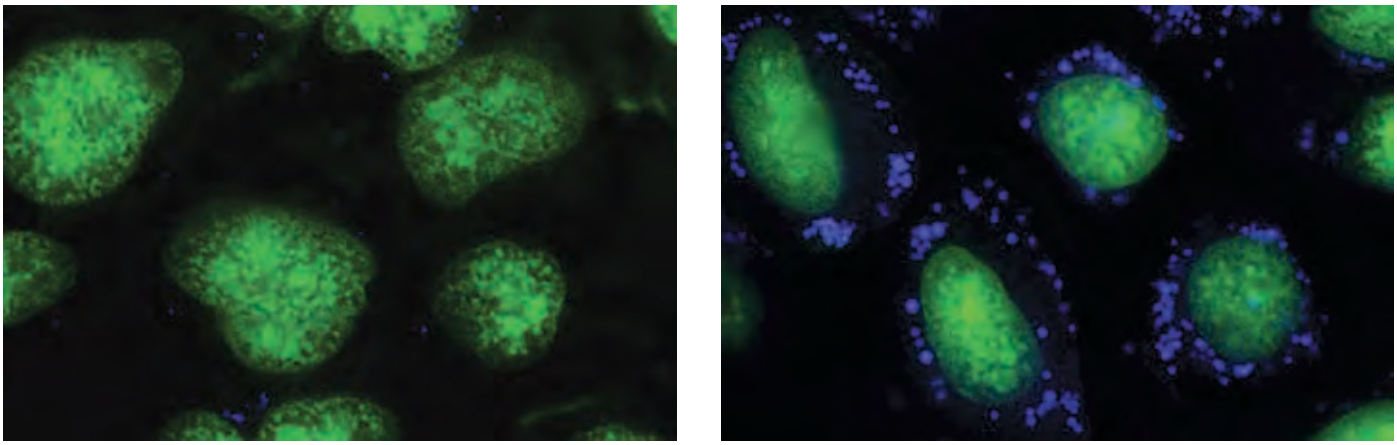

Figure 2.5 Autophagy Super Blue™ labeled vesicles were induced by starvation in HeLa cells. HeLa cells were incubated in a regular DMEM medium (Left: Control) or in 1X HBSS buffer with 5% serum (Right: Autophagy Treatment, Cat#23001) for 16 hours. Both control and treated cells were incubated with Autophagy Super Blue™ working solution for 20 minutes in a 37 °C, 5% CO_2 incubator, and washed 3 times with wash buffer. Cells were imaged immediately under a fluorescence microscope with a DAPI channel (Blue). Cell nuclei were stained with Nuclear Green™ LCS1 (Green, Cat#17540).

microbial infection and disease.

Cell Meter™ autophagy fluorescence imaging kit utilizes our proprietary Autophagy Super Blue™ probe, to selectively target and analyze autophagy activity. Autophagy Super Blue™ has a maximum fluorescence excitation/emission at 333/518 nm. This assay has been optimized for the direct detection of autophagy in both detached and attached cells. It includes a robust protocol and all the essential components to successfully detect autophagy activity. Additionally, our Cell Meter™ autophagy fluorescence imaging kit is well-suited for fluorescence microscopy and flow cytometry.

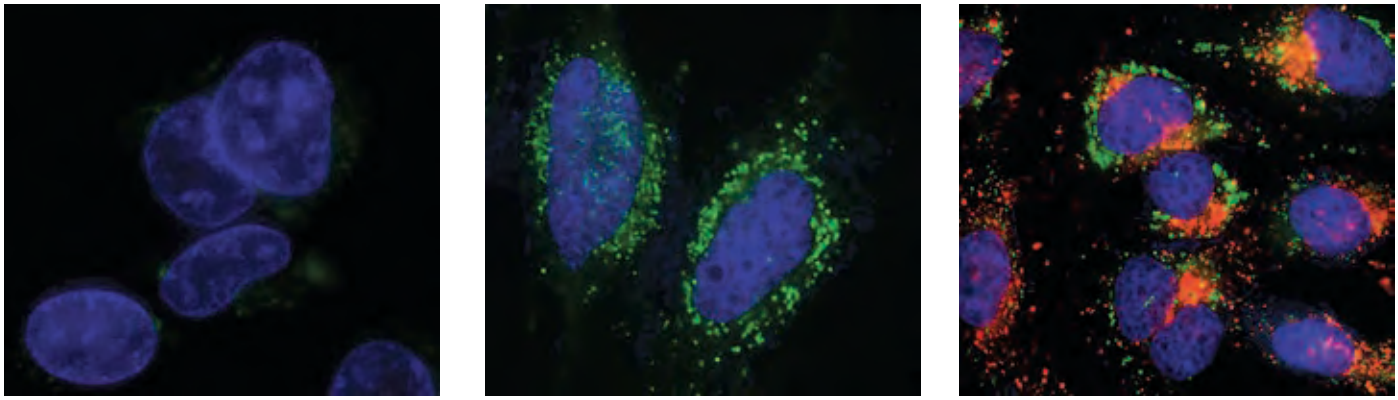

Figure 2.6 Autophagy Green™ labeled vesicles were induced by starvation in HeLa cells. HeLa cells were incubated in a regular DMEM medium (Left: Control) or in 1X HBSS buffer with 5% serum (Middle: Autophagy Treatment, Cat#23002) for 16 hours. Both control and starved cells were incubated with Autophagy Green™ working solution for 20 minutes in a 37 °C, 5% CO_2 incubator, and then washed 3 times with wash buffer. Cells were imaged immediately under a fluorescence microscope with a FITC channel (Green). Cell nuclei were stained with Hoechst 33342 (Blue, Cat#17530) Cell lysosomes were stained with LysoBrite™ Orange (Right, Orange, Cat#22657).

Product Ordering Information For Lysosome and Autophagy Staining Probes and Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
23000	Cell Meter™ Autophagy Assay Kit *Blue Fluorescence*	200 Tests	333	518
23002	Cell Meter™ Autophagy Assay Kit *Green Fluorescence*	200 Tests	447	553
23001	Cell Meter™ Autophagy Fluorescence Imaging Kit	200 Tests	333	518
22655	Cell Navigator™ Lysosome Staining Kit *Blue Fluorescence*	1 kit	433	480
22659	Cell Navigator™ Lysosome Staining Kit *Deep Red Fluorescence*	1 kit	596	619
22651	Cell Navigator™ Lysosome Staining Kit *Green Fluorescence with 405 nm Excitation*	1 kit	405	505
22656	Cell Navigator™ Lysosome Staining Kit *Green Fluorescence*	1 kit	445	503
22652	Cell Navigator™ Lysosome Staining Kit *NIR Fluorescence*	1 kit	636	650
22657	Cell Navigator™ Lysosome Staining Kit *Orange Fluorescence*	1 kit	542	556
22658	Cell Navigator™ Lysosome Staining Kit *Red Fluorescence*	1 kit	575	597
22642	LysoBrite™ Blue	500 Tests	433	480
22646	LysoBrite™ Deep Red	500 Tests	596	619
22643	LysoBrite™ Green	500 Tests	445	503
22641	LysoBrite™ NIR	500 Tests	636	650
22644	LysoBrite™ Orange	500 Tests	542	556
22645	LysoBrite™ Red	500 Tests	575	597

Nuclei
Labeling Probes and Kits

The nucleus is a membrane-enclosed organelle found in eukaryotic cells containing the cell's genetic material. This genetic material, in the form of multiple long linear DNA molecules, is organized into complex thread-like structures known as chromosomes. Chromosomes house genes which comprise of the cell's nuclear genome and are structured in such a way to promote cell functionality. A key function of the nucleus is to maintain the integrity of these genes and through regulating gene expression, control the activities of the cell. The nuclear envelope, an impermeable double membrane enclosing the entire organelle, and the nuclear matrix are the main structural components making up the nucleus. Large transmembrane protein complexes known as nuclear pores facilitate and regulate nuclear transport of molecules across the nuclear envelope. Movement of large molecules such as proteins and RNA through these pores is essential for gene expression and maintenance of chromosomes. Application of nucleic acid stains can be used to investigate cell viability, proliferation and apoptosis.

Nuclei Labeling of Live Cells

Hoechst 33258 and Hoechst 33342 are cell membrane-permeant, minor groove-binding DNA stains. These relatively nontoxic, water soluble stains exhibit a bright blue fluorescence upon binding to DNA. Compared to Hoechst 33258, Hoechst 33342 displays slightly higher membrane permeability. Both dyes can be excited by the UV spectral line around 350 nm with an emission maximum at 461 nm. This relatively large Stokes shifts makes Hoechst stains suitable for multicolor labeling experiments.

DAPI (4′, 6-diamidino-2phenylindole) is a cell permeable, fluorescent stain exhibiting good water solubility. Its strong affinity for the A-T regions in DNA makes it an excellent nuclear counterstain for labeling and visualizing nuclei of live cells in fluorescence microscopy and flow cytometry. DAPI demonstrates blue fluorescence upon binding to double-stranded DNA (dsDNA) with an excitation/emission maximum of 358/461 nm. DAPI will also bind to RNA. However, it is not as strongly fluorescent because its emission shifts to around 500 nm. DAPI's blue emission is convenient for multiplexing assays because of the minimal spectral overlap between DAPI and green- or red- fluorescent stains such as GFP or Texas Red®. Additionally, DAPI is used for the detection of mycoplasma or virus DNA in cell cultures.

LDS 751 is a fluorescent cell-permeant nucleic acid stain that can be well excited with the 488 nm laser line, although it has a peak excitation at ~543 nm when bound to dsDNA. LDS-751's large Stokes shift, emission maximum at 712 nm, makes it well suited for multicolor analysis. Upon binding to dsDNA, LDS 751 exhibits a ~20-fold fluorescence enhancement. LDS 751 has been used to discriminate intact nucleated cells from non-nucleated and damaged nucleated cells. In flow cytometry, LDS 751 can be used to identify distinct cell types in complex populations comprised of neutrophils, leukocytes and monocytes.

AAT Bioquest offers a family of fluorogenic, DNA-selective and cell-permeant dyes for analyzing DNA content in living cells. Our Nuclear Green™ LCS1, Nuclear Orange™ LCS1, Nuclear Red™ LCS1 and Nuclear Yellow dyes have their respective fluorescent colors significantly enhanced upon binding to DNA. Nuclear Green™ LCS1 has an excitation/emission maximum at 503/523 nm. Nuclear Orange™ LCS1 has an excitation/emission maximum at 514/555 nm. Nuclear Red™ LCS1 has an excitation/emission maximum at 622/645 nm. Nuclear Yellow has an excitation/emission maximum at 355/495 nm. They are well-suited for use in various applications

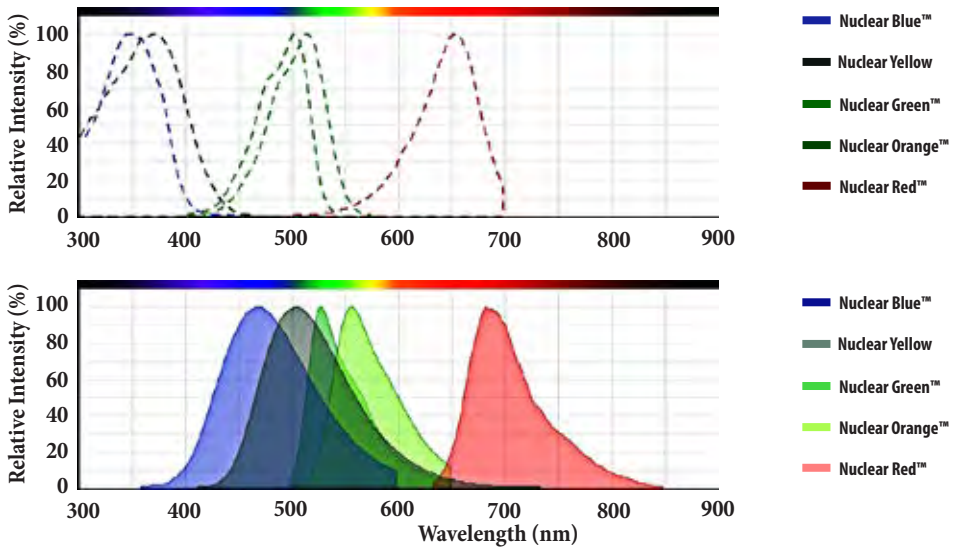

Figure 3.1 Top image is excitation spectra of Nuclear Dyes. Bottom image is emission spectra of Nuclear Dyes.

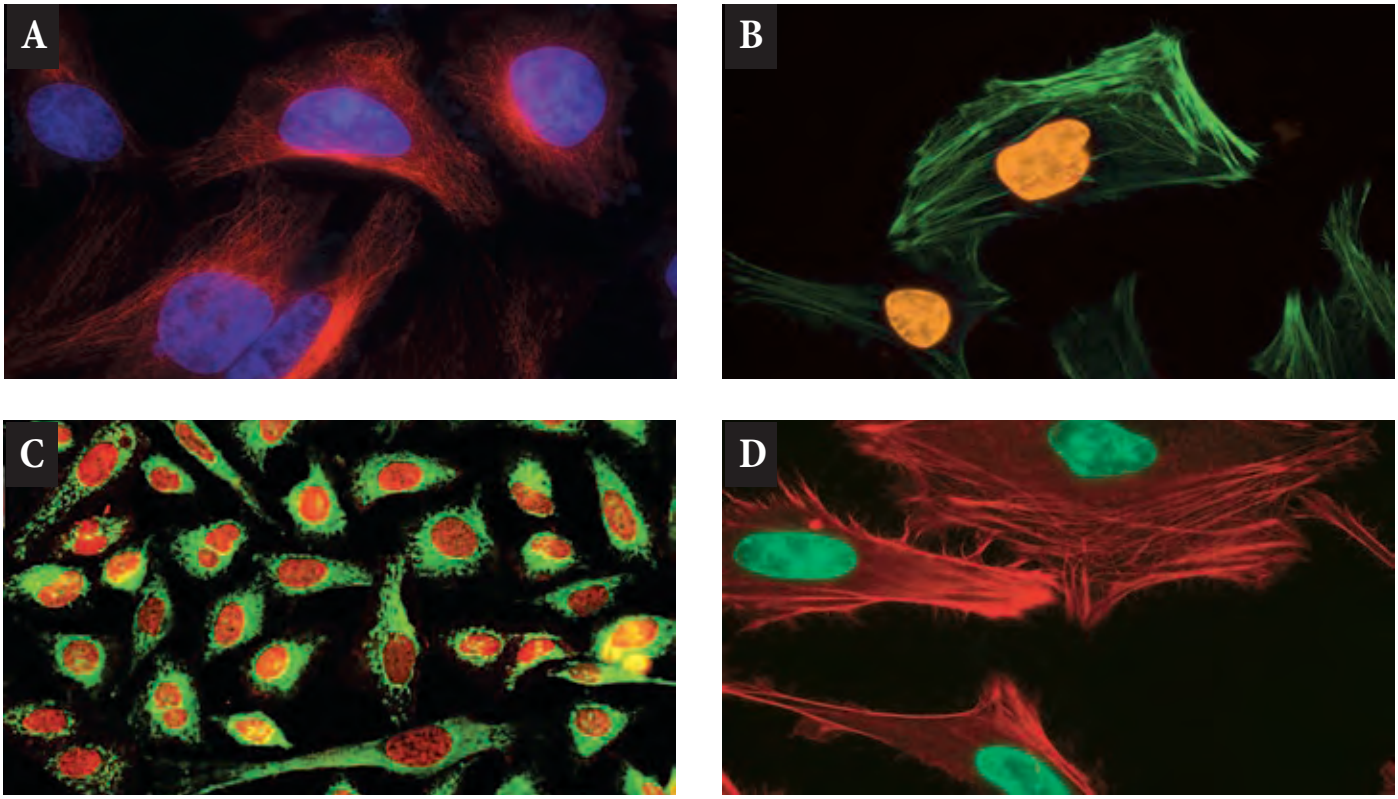

Figure 3.2 Four-panel composite image of HeLa cells stained with Nuclear labeling probes. **A**) Image of HeLa cells stained with mouse anti-tubulin followed with iFluor™ 594 goat anti-mouse IgG (H+L) (Red, Cat#16548); and nuclei were co-stained with nuclei stain Nuclear Blue™ DCS1 (Blue, Cat#17548). **B**) Image of HeLa cells stained with Phalloidin-iFluor™ 488 Conjugate (Green, Cat#23115); and nuclei were co-stained with nuclei stain Nuclear Orange™ DSC1 (Orange, Cat#17551). **C**) Image of HeLa cells stained with MitoLite™ Green FM (Green, Cat#22695); and nuclei were co-stained with nuclei stain Nuclear Red™ LCS1 (Red, Cat#17542). **D**) Image of HeLa cells stained with Phalloidin-iFluor™ 647 Conjugate (Red, Cat#23127); and nuclei were co-stained with nuclei stain Nuclear Green™ DCS1 (Green, Cat#17550).

such as fluorescence imaging, microplate assays and flow cytometry. Various combinations of these DNA-binding dyes may be used for multicolor analysis of live cells with proper filter sets.

Nuclei Labeling of Dead Cells

Propidium iodide (PI) is a cell-impermeable, fluorescent intercalating agent belonging to the same chemical class as ethidium bromide (EtBr). As in the case of EtBr, propidium iodide's fluorescence is enhanced 20-30 fold upon binding to nucleic acids. The fluorescence excitation maximum is red-shifted by 30-40 nm while its fluorescence emission maximum is blue-shifted by ~15 nm. Like DAPI, PI can bind to DNA, requiring treatment with nucleases to distinguish between RNA and DNA staining. PI is well-adapted for various applications such as fluorescence microscopy and flow cytometry. In flow cytometry, PI is used as a DNA stain to evaluate cell viability or DNA content in cell cycle analysis. In fluorescence microscopy, PI can be used to visualize the nucleus and other DNA-containing organelles. Additionally, PI is used to differentiate necrotic, apoptotic and normal cells.

7-Amino actinomycin D (7-AAD) is another non-permeant dye useful at identify non-viable cells. Cells with damaged plasma membranes or with impaired cell metabolism are unable to prevent the dye from entering the cell. Once inside the cell, it binds to intracellular DNA producing highly fluorescent adducts which identify the cell as "non-viable". 7-AAD is well-adapted for flow cytometry. It is excited by the 488 nm laser line of an argon laser with fluorescence detected above 650 nm. Although 7-AAD's emission intensity is lower than that of PI, the longer wavelength emission makes it more adequate for multiplexing assays in combination with other 488 nm-excited fluorochromes such as FITC and PE.

AAT Bioquest offers a family of fluorogenic, DNA-selective and cell-impermeant dyes for analyzing DNA content in dead, fixed, or apoptotic cells. Our Nuclear Green™ DCS1, Nuclear Orange™ DCS1 and Nuclear Red™ DCS1 dyes have their respective fluorescent colors significantly enhanced upon binding to DNA. Nuclear Green™ DCS1 has an excitation/emission maximum at 503/526 nm. Nuclear Orange™ DCS1 has an excitation/emission maximum at 528/576 nm. Nuclear Red™ DCS1 has an excitation/emission maximum at 642/660 nm. They are well-suited for use in various applications such as fluorescence imaging, microplate assays and flow cytometry. Various combinations of these DNA-binding dyes may be used for multicolor analysis of dead cells.

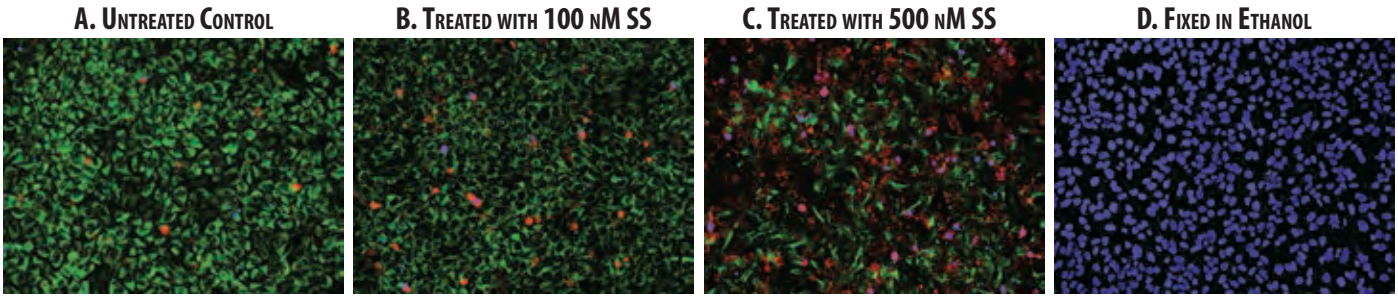

Figure 3.3 Fluorescence images of HeLa cells labeled with Cell Meter™ Multiplexing Live, Apoptotic and Necrotic Detection Kit (Cat#22846). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 0-500 nM staurosporine (SS) at 37 °C for 4 hours (**A**-**C**), or fixed in ethanol (**D**), then incubated with triple fluorescence assay solution for 1 hour. The fluorescence signal was measured using a fluorescence microscope with TRITC filter for healthy cells (shown as Green, a pseudo color for Orange), Cy5 filter for apoptotic (Red) and DAPI filter for necrotic cells (Blue), respectively.

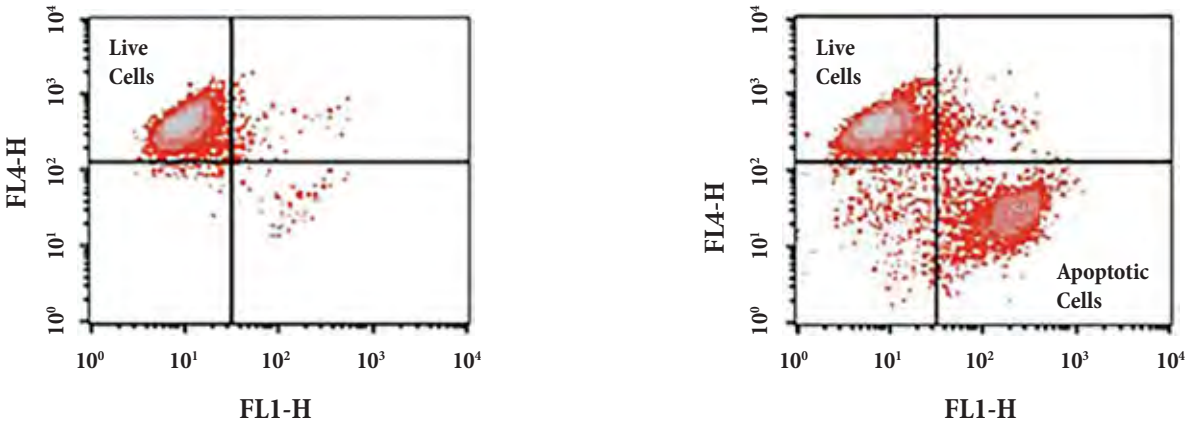

Figure 3.4 Increase in fluorescence intensity of Nuclear Green™ DCS1 (Cat#17550) with the addition of camptothecin in Jurkat cells. Jurkat cells were treated overnight without (Left) or with 20µM camptothecin (Right) in a 37°C, 5% CO_2 incubator, and then dye loaded with Nuclear Green DCS1 for 60 minutes. At the end of 15 minutes of Nuclear Green™ DCS1 dye loading, MitoLite™ NIR (Cat#22802) was added for multicolor analysis. The fluorescence intensity of Nuclear Green™ DCS1 and MitoLite™ NIR was measured with a FACSCalibur flow cytometer using FL1 channel and FL4 channel.

Product Ordering Information For Nucleus Labeling Probes and Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
17501	7-AAD [7-Aminoactinomycin D] *CAS 7240-37-1*	1 mg	546	647
17510	DAPI [4,6-Diamidino-2-phenylindole, dihydrochloride] *CAS 28718-90-3*	10 mg	358	461
17520	Hoechst 33258 *CAS 23491-45-4*	100 mg	352	461
17530	Hoechst 33342 *CAS 23491-52-3*	100 mg	350	461
17537	Hoechst 34580 *CAS 911004-45-0*	5 mg	368	437
17561	LDS 751 *CAS 181885-68-7*	25 mg	543	712
17548	Nuclear Blue™ DCS1	0.5 mL	350	461
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	642	660
17542	Nuclear Red™ LCS1	0.5 mL	622	645
17545	Nuclear Red™ LCS2	0.5 mL	651	681
17543	Nuclear Violet™ LCS1	0.5 mL	401	459
17539	Nuclear Yellow [Hoechst S769121] *CAS 74681-68-8*	25 mg	355	495
17515	Propidium iodide *CAS 25535-16-4*	25 mg	535	617
22630	Cell Navigator™ Live Cell RNA Imaging Kit *Green Fluorescence*	100 Tests	503	511
17611	StrandBrite™ Green RNA Quantifying Reagent *200X DMSO Solution*	10 mL	500	525
17656	StrandBrite™ Green Fluorimetric RNA Quantitation Kit	1 Kit	500	525
17657	StrandBrite™ Green Fluorimetric RNA Quantitation Kit *High Selectivity*	1 Kit	508	528
17655	StrandBrite™ Green Fluorimetric RNA Quantitation Kit *Optimized for Microplate Readers*	1 Kit	500	525
17610	StrandBrite™ Green RNA Quantifying Reagent *200X DMSO Solution*	1 mL	500	525

ER, Plasma Membrane and Lipid Droplets Labeling Probes and Kits

Staining Endoplasmic Reticulum

The endoplasmic reticulum (ER) is an organelle found in all eukaryotic cells. It consists of an interconnected network of membrane-enclosed tubules and flattened sacs that extends from the nuclear membrane throughout the cytoplasm. The rough ER which faces outward towards the cytosol is coated with membrane bound ribosomes responsible for protein synthesis. The smooth ER lacks these ribosomes. Its key functions are lipid manufacture and metabolism, production of steroid hormones, and detoxification. Together these ER complexes are responsible for the translocation of proteins across the ER membrane, synthesis of phospholipids and steroids, as well as storage of calcium ions in the ER and their regulated release into the cytosol.

AAT Bioquest has developed two distinct cell permeant probes, ER Green™ and ER Red™, for staining live cell ER in a wide variety of mammalian cell types. As key components in our Cell Navigator™ Live Cell Endoplasmic Reticulum Staining Kits, they provide excellent and rapid staining with high selectivity for ER over other cellular compartments such as mitochondria. ER Green™ and ER Red™ probes exhibit excitation/emission maxima of 503/511 nm and 589/620 nm, respectively. The fluorescence staining in live cell ER can be maintained after fixation with formaldehyde, enabling further multi-color staining. In addition, these kits can be adapted for various applications such as fluorescence microscopy, microplate assays and flow cytometry.

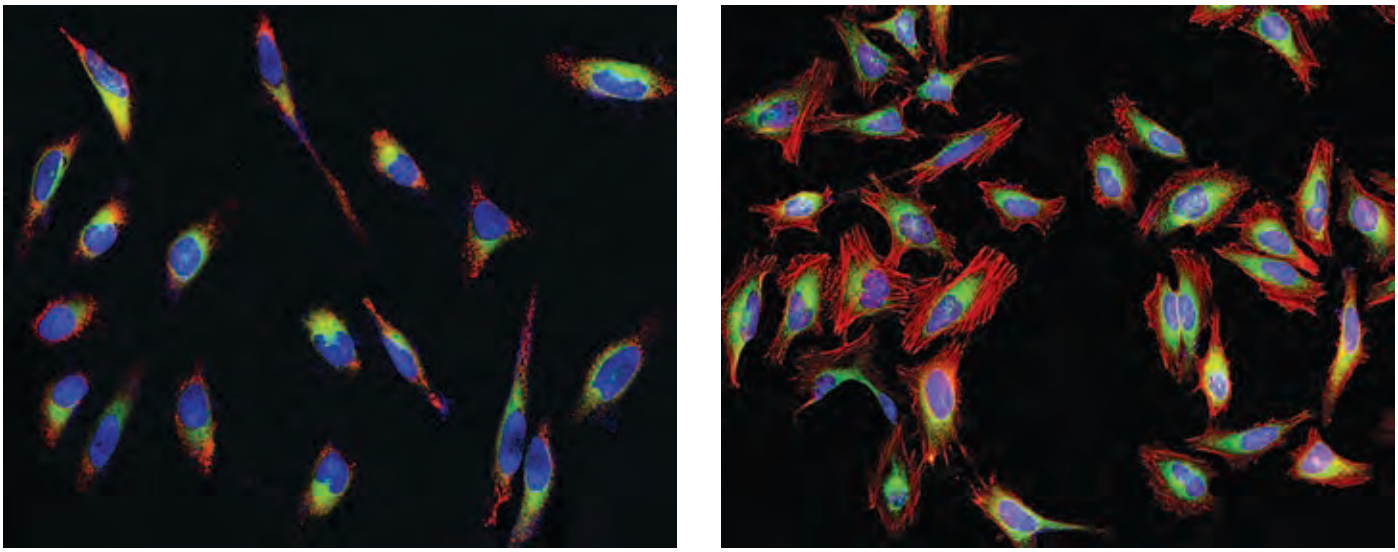

Figure 4.1 Fluorescence images of endoplasmic reticulum (ER) staining in HeLa cells cultured in a 96-well black-wall clear-bottom plate using fluorescence microscope with a FITC filter set. Left: Live cells were stained with ER-selective probe ER Green™ (Green, Cat#22635), mitochondria dye MitoLite™ Red FX600 (Red, Cat#22677) and nuclei stain Hoechst 33342 (Blue, Cat#17530). Right: Live cells stained with ER Green™ (Green, Cat22635) were fixed with 4% formaldehyde, and labeled with F-actin dye iFluor™ 594-Phalloidin (Red, Cat#23122) and nuclei stain DAPI (Blue, Cat#17507).

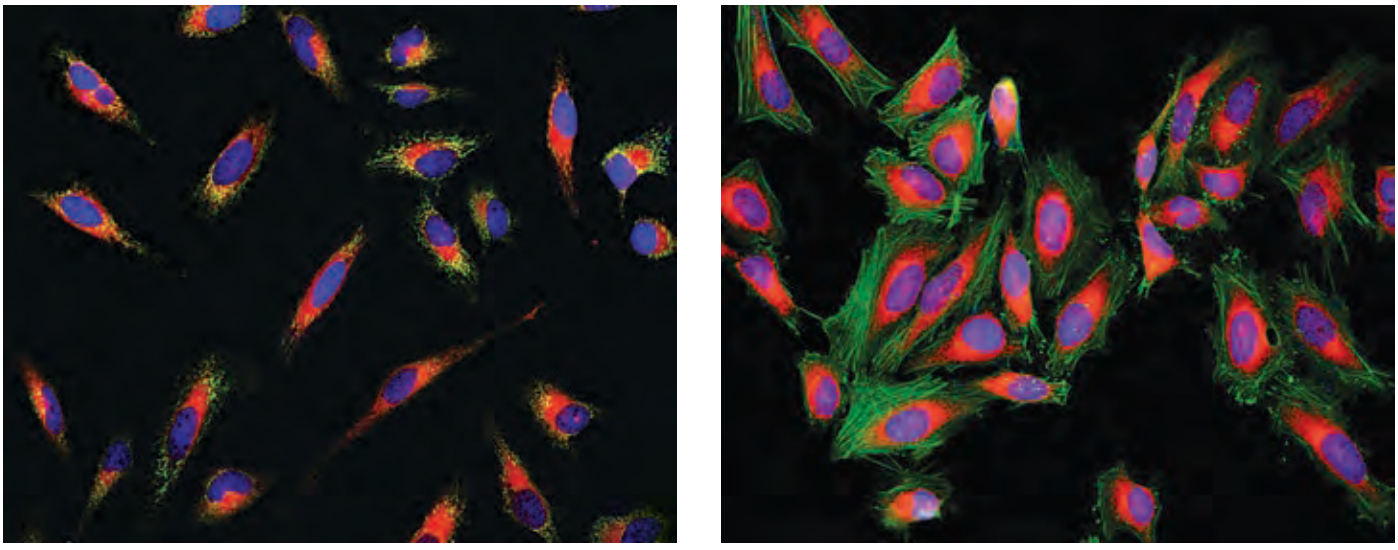

Figure 4.2 Fluorescence images of endoplasmic reticulum (ER) staining in HeLa cells cultured in a 96-well black-wall clear-bottom plate using fluorescence microscope with a TRITC filter set. Left: Live cells were stained with ER-selective probe ER Red™ (Red, Cat#22636), mitochondria dye MitoLite™ Green (Green, Cat#22675) and nuclei stain Hoechst 33342 (Blue, Cat#17530). Right: Live cells stained with ER Red™ (Red, Cat#22636) were fixed with 4% formaldehyde, then labeled with F-actin dye iFluor™ 488-Phalloidin (Green, Cat#22661,) and nuclei stain DAPI (Blue, Cat#17507).

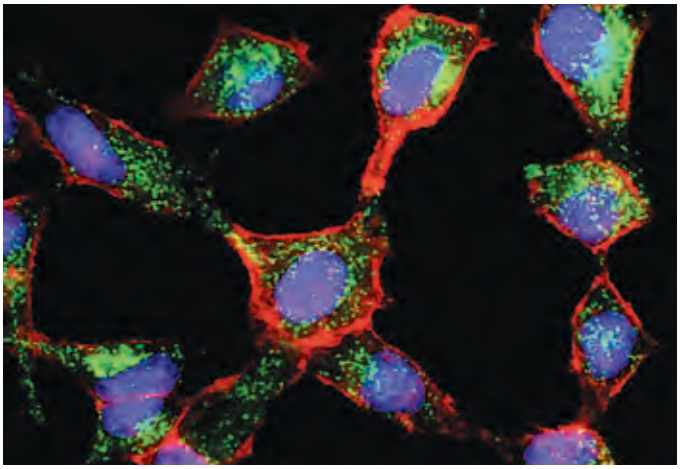

Figure 4.3 Fluorescence images of HeLa cells co-stained with mitochondria dye MitoLite™ Green (Green, Cat#22695), Cellpaint™ Deep Red (Red, Cat#22681) to label plasma membrane, and nuclei stain Nuclear Blue™ DCS1 (Blue, Cat#17548). The cells were fixed in 4% formaldehyde.

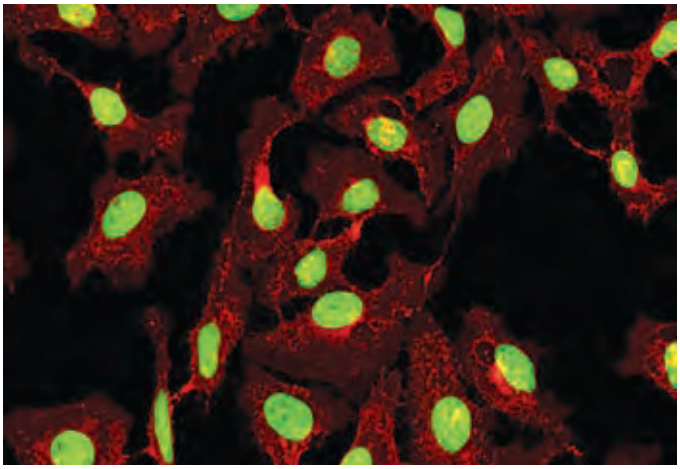

Figure 4.4 Fluorescence image of HeLa cells co-stained with Cellpaint™ Orange (Red, Cat#22680) to label plasma membrane, and nuclei stain Nuclear Green™ DCS1 (Green, Cat#17550). The cells were fixed in 4% formaldehyde.

Staining Plasma Membrane

The cell membrane or plasma membrane is a thin semi-permeable membrane separating the interior of all cells from their environment. It consists of a phospholipid bilayer embedded with proteins. The plasma membrane is cell permeable to ions and organic molecules facilitating their movement in and out of the cell. It is involved in a variety of key cellular processes such as cell adhesion, ion conductivity and cell signaling. Additionally, the plasma membrane serves as an anchoring site for several extracellular structures such as the cell wall, glycocalyx and intracellular cytoskeleton.

Cellpaint™: Plasma Membrane Labeling Probes

AAT Bioquest has developed three distinct probes, Cellpaint™ Orange, Cellpaint™ Deep Red and Cellpaint™ TSP for uniformly staining cell membranes in a wide variety of mammalian cells. As key components in our Cell Navigator™ Cell Membrane Staining Kits, they provide an excellent tool for rapidly staining the plasma membrane in suspended or attached live cells. Cellpaint™ Orange and Cellpaint™ Deep Red exhibit excitation/emission maxima of 555/575 nm and 650/670 nm, respectively. The fluorescence staining in cell membranes can be maintained after fixation with formaldehyde, enabling further multi-color staining. Additionally, these kits are well suited for various applications such as fluorescence imaging and flow cytometry.

"Cellpaint™ TSP is a styryl pyridine-based fluorescent membrane probe utilized for imaging the plasma membrane in living cells and tissues. TSP probes are molecular rotors whose fluorescence significantly enhances when applied in viscous media. Its fluorescence is also microenvironment-sensitive, enabling turn-on imaging of plasma membranes with a high signal-to-background ratio as reported by Guo et al. 2016 . In addition, Guo et al. 2016 demonstrated that TSP has high photostability, low cytotoxicity, and high degree of biocompatibility. TSP plasma membrane probes can also be used for two photon bioimaging.

Staining Lipids

Lipid droplets or lipid bodies are lipid-rich cellular organelles regulating the storage and hydrolysis of neutral lipids. They are located mostly in the adipose tissue of all eukaryotic organisms and serve as a lipid reservoir for many different processes. Such processes include fatty acid and cellular cholesterol for energy as well as membrane formation and maintenance. Abnormal accumulation of the cytoplasmic lipid droplets have been linked to a variety of pathological conditions and can be an indicator of metabolic deficiency or pathogenesis.

AAT Bioquest has developed two distinct lipophilic stains, Nile Green™ and Droplite™ Red. As key components in our Cell Navigator™ Fluorimetric Lipid Droplets Assay Kits, they are robust tools for quantitatively measuring lipid droplet accumulation. Nile Green™ and Droplite™ Red lipophilic stains exhibit excitation/emission maxima of 485/520 nm and 550/640 nm, respectively. Both stains are intensely fluorescent in lipid-rich environments while exhibiting minimal fluorescence in aqueous media. They are well-suited for the detection of intracellular lipid droplets across various platforms such as fluorescence microscopy, flow cytometry and fluorescence microplate readers. The green and red fluorescence signals of Nile Green™ and Droplite™ Red can be observed using the filter set of FITC and TRITC, respectively.

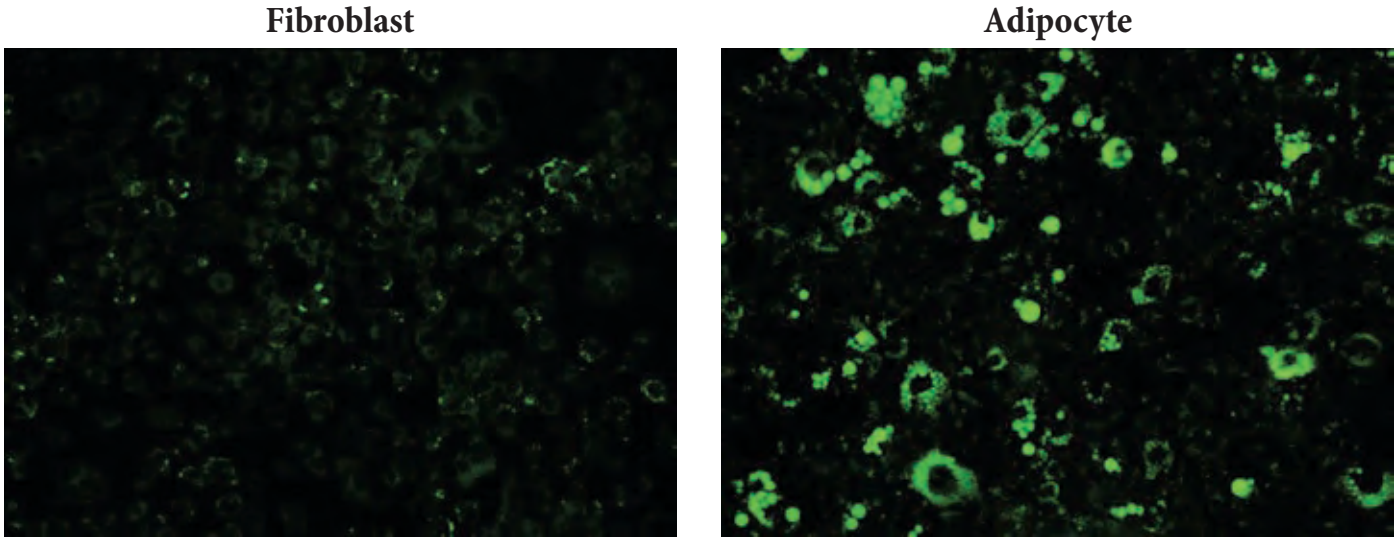

Figure 4.5 Fluorescence images of intracellular lipid droplets in 3T3-L1 Fibroblast (Left) and Adipocyte cells (Right) using Cell Navigator™ Lipid Droplets Fluorescence Assay Kit (Cat#22730). The fluorescence signal was measured using fluorescence microscope with a FITC filter.

Product Ordering Information For ER, Plasma Membrane and Lipid Droplet Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (nm)
22680	Cell Navigator™ Cell Plasma Membrane Staining Kit *Orange Fluorescence*	500 Tests	555	575
22681	Cell Navigator™ Cell Plasma Membrane Staining Kit *Red Fluorescence*	500 Tests	650	670
22682	CellPaint™ TSP Membrane Stain	500 Tests	488	645
22730	Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit *Green Fluorescence*	500 Tests	550	640
22735	Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit *Red Fluorescence*	500 Tests	550	640
22635	Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit *Green Fluorescence*	100 Tests	503	511
22636	Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit *Red Fluorescence*	100 Tests	589	620
22033	DiD labeling solution [1,1-Dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine] *5 mM DMSO solution*	10 mL	644	663
22101	DiI iodide [1,1-Dioctadecyl-3,3,3,3- tetramethylindocarbocyanine iodide]	100 mg	549	565
22102	DiI perchlorate [1,1-Dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate] *CAS 41085-99-8*	100 mg	549	565
22103	DiI triflate [1,1-Dioctadecyl-3,3,3,3-tetramethylindocarbocyanine triflate]	100 mg	549	565
22056	DiIC1(5) iodide [1,1,3,3,3,3-Hexamethylindodicarbocyanine iodide]	25 mg	638	658
22035	DiIC12(3) perchlorate [1,1-Didodecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate]	25 mg	549	565
22050	DiIC12(3)-DS [1,1-Diododecyl-3,3,3,3-tetramethylindocarbocyanine-5,5-disulfonic acid]	5 mg	555	570
22051	DiIC12(5)-DS [1,1-Diododecyl-3,3,3,3-tetramethylindodicarbocyanine-5,5-disulfonic acid]	5 mg	650	670
22044	DiIC16(3) perchlorate [1,1-Dihexadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate]	25 mg	549	565
22052	DiIC18(3)-DS [1,1-Dioctadecyl-3,3,3,3-tetramethylindocarbocyanine-5,5-disulfonic acid]	5 mg	555	570
22054	DiIC18(5)-DS [1,1-Dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine-5,5-disulfonic acid]	5 mg	650	670
22066	DiO perchlorate [3,3-Dioctadecyloxacarbocyanine perchlorate]	25 mg	484	501
22042	DiOC16(3) perchlorate [3,3-Dihexadecyloxacarbocyanine perchlorate]	25 mg	484	501
22038	DiOC2(3) iodide [3,3-Diethyloxacarbocyanine iodide]	25 mg	482	497
22039	DiOC3(3) iodide [3,3-Dipropyloxacarbocyanine iodide]	25 mg	482	497
22045	DiOC5(3) iodide [3,3-Dipentyloxacarbocyanine iodide]	25 mg	482	504
22046	DiOC6(3) iodide [3,3-Dihexyloxacarbocyanine iodide]	25 mg	482	504
22040	DiOC7(3) iodide [3,3-Diheptyloxacarbocyanine iodide]	25 mg	482	504
22070	DiR iodide [1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide]	25 mg	748	780
22073	DiSC2(3) [3,3-Diethylthiacarbocyanine iodide]	25 mg	560	571
22077	DiSC2(7) [3,3-Diethylthiatricarbocyanine iodide] *CAS#: 3071-70-3*	25 mg	770	790
22076	DiSC3(5) [3,3-Dipropylthiadicarbocyanine iodide]	25 mg	660	675
22190	Nile Red *CAS#: 7385-67-3*	25 mg	552	636

F-Actin (Phalloidin) Labeling Probes and Kits

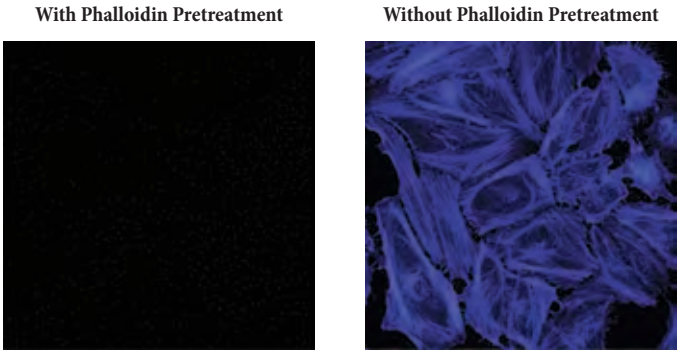

Figure 5.1 Fluorescence images of HeLa cells fixed with 4% formaldehyde then stained with Cell Navigator™ F-Actin Labeling Kit *Blue Fluorescence* (Cat#22660) in a Costar black 96-well plate. Cells were labeled with iFluor™ 350-Phalloidin (Blue, Cat#23110) with (left) or without (right) pre-treatment of phalloidin for 10 minutes.

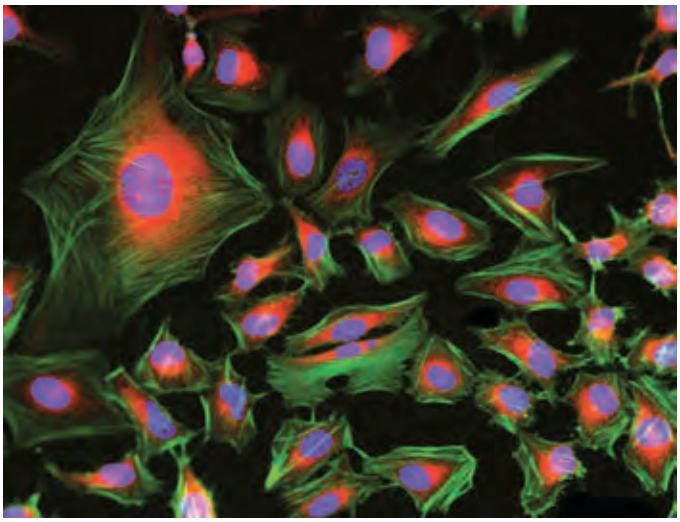

Figure 5.2 Fluorescence image of HeLa cells stained with Phalloidin-iFluor™ 488 Conjugate (Green, Cat#23115) using fluorescence microscope with a FITC filter set (Green). The cells were fixed in 4% formaldehyde, co-labeled with mitochondria dye MitoLite™ Red FX600 (Red, Cat#2677) and Nuclear Blue™ DCS1 (Blue, Cat#17548).

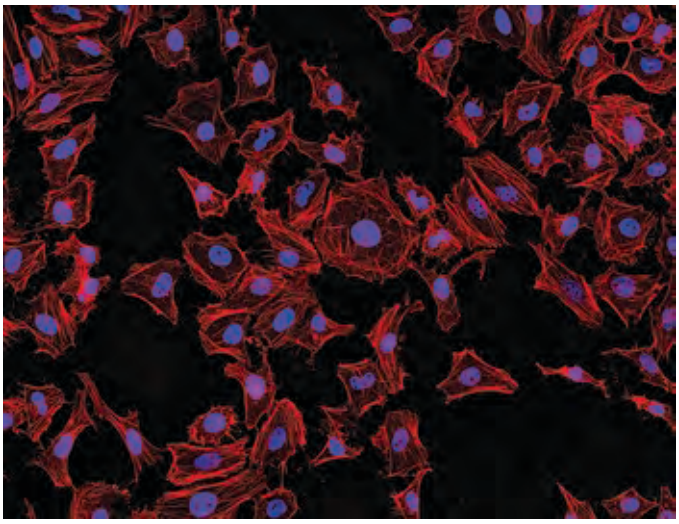

Figure 5.3 Fluorescence image of HeLa cells stained with Phalloidin-iFluor™ 514 (Cat#23116) Conjugate using fluorescence microscope with a TRITC filter set (Red). Fixed cells were counterstained with Nuclear Blue™ DCS1 (Blue, Cat#17548).

Staining F-Actin (Phalloidin)

Actin is a globular multi-functional protein, roughly 42-kDa in mass and can be found in almost all eukaryotic cells. Actin is the monomeric subunit of two types of filaments in cells: microfilaments and thin filaments. Microfilaments are one of the three major components of the cytoskeleton while thin filaments are part of the contractile structure in muscle cells. Actin may present itself as either G (globular)-actin or F (filament)-actin both of which are crucial for cellular functionality. Actin participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, as well as the establishment and maintenance of cell junctions and cell shape.

AAT Bioquest offers a selection of fluorescent phalloidin derivatives in various distinct colors for multicolor imaging of F-actin. These fluorescent derivatives are suited for localizing actin filaments in living or fixed cells, as well as visualizing individual actin filaments *in vitro*. They are used as an important tool in investigating actin networks at high resolution. When used at nanomolar concentrations, phalloidin derivatives act as probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments.

Compared to actin monomers, phalloidin tightly binds to actin filaments decreasing the rate constant for the dissociation of actin subunits from filament ends. This stabilizes actin filaments by preventing filament depolymerization. Moreover, phalloidin is found to inhibit the ATP hydrolysis activity of F-actin. It functions differently at various concentrations in cells. At low concentrations, phalloidin recruits the less polymerized forms of cytoplasmic actin and filamin forming stable "islands" of aggregated actin polymers without interfering with stress fibers.

AAT Bioquest's Cell Navigator™ F-Actin Labeling Kits are a set of fluorescence imaging tools optimized for labeling F-actins in fixed cells. Each kit contains a distinct iFluor-phalloidin conjugate that selectively binds to F-actins and exhibits either a blue, green, orange or red fluorescence. These high-affinity iFluor-phalloidin probes conveniently label, identify and quantify F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. Each kit includes all the essential components with a robust staining protocol that requires minimal hands-on time.

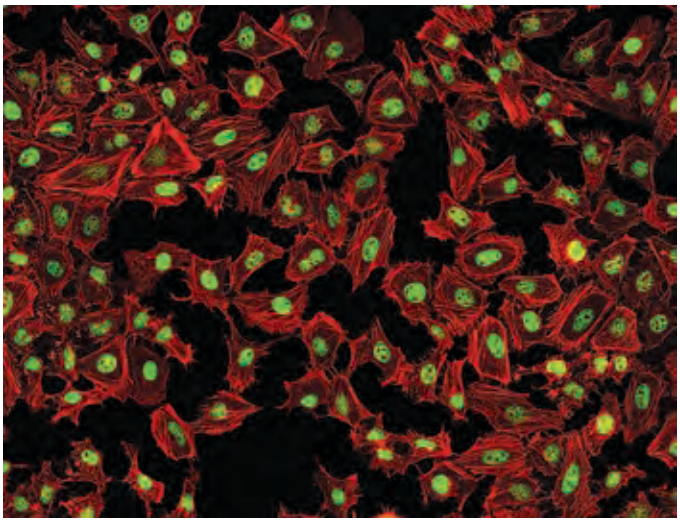

Figure 5.4 Fluorescence image of HeLa cells stained with Phalloidin-iFluor™ 532 Conjugate (Cat#23117) using fluorescence microscope with a TRITC filter set (Red). Fixed cells were counterstained with Nuclear Green™ DCS1 (Green, Cat#17550).

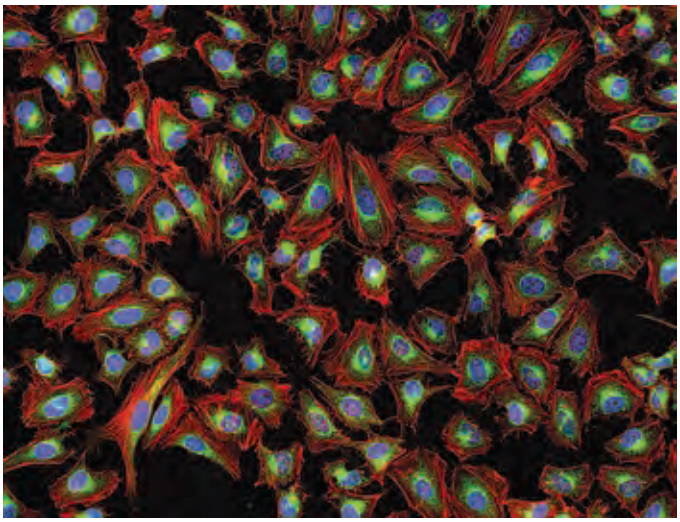

Figure 5.5 Fluorescence image of HeLa cells stained with Phalloidin-iFluor™ 647 Conjugate (Red, Cat#23127) using fluorescence microscope with a Cy5 filter set (Red). Live cells were first stained with mitochondrial dye MitoLite™ Green (Green, Cat#22675). After fixation in 4% formaldehyde, cells were labeled with Phalloidin-iFluor™ 647 and counterstained with Nuclear Blue™ DCS1 (Blue, Cat#17548).

Product Ordering Information For Phalloidin-iFluor™ Conjugates and F-Actin Labeling Kits

Cat #	Product Name	Unit Size	Excitation (nm)	Emission (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
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

Alphabetical Index

Catalog Number Index

Notes

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Website: http://www.biomol.de

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Website: http://www.assaymatrix.com

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

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Website: http://www.tjbiolite.com

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Website: http://www.izinta.hu

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Email: info@biosite.se
Website: http://www.biosite.se

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Email: info@bcls.in
Website: http://www.bcls.in

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Website: http://www.genxbio.com

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Website: http://www.advansys.co.il

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Website: http://www.spacesrl.com

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Website: http://www.valterocchiena.com

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Website: http://www.cosmobio.co.jp

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Website: http://www.nacalai.com

Wako Pure Chemical Industries, Ltd.
Email: labchem-tec@wako-chem.co.jp
Website: http://www.wako-chem.co.jp

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Email: cms@cmscorp.co.kr
Website: http://www.cmscorp.co.kr

Kimnfriends Corporation
Email: kimnfriends@hanmail.net
Website: http://www.kimnfriends.co.kr

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

Netherlands:
ITK Diagnostics BV
Email: info@itk.nl
Website: http://www.itk.nl

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

Poland and Slovenia:
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Website: http://www.biomol.de

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

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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

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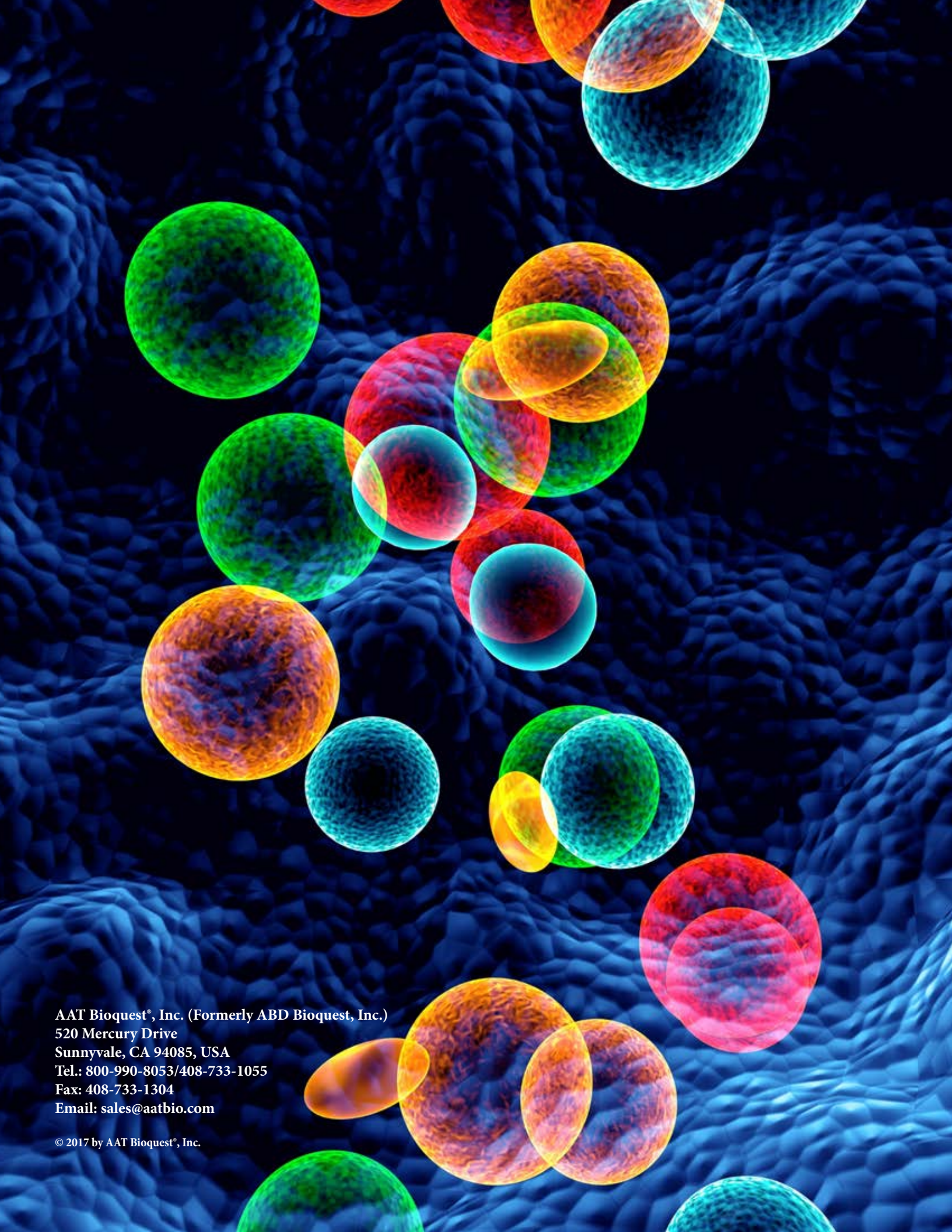

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