Cell Apoptosis & Proliferation

APOPTOSIS · CYTOTOXICITY · PROLIFERATION

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AAT Bioquest®
Advancing Assay & Test Technologies

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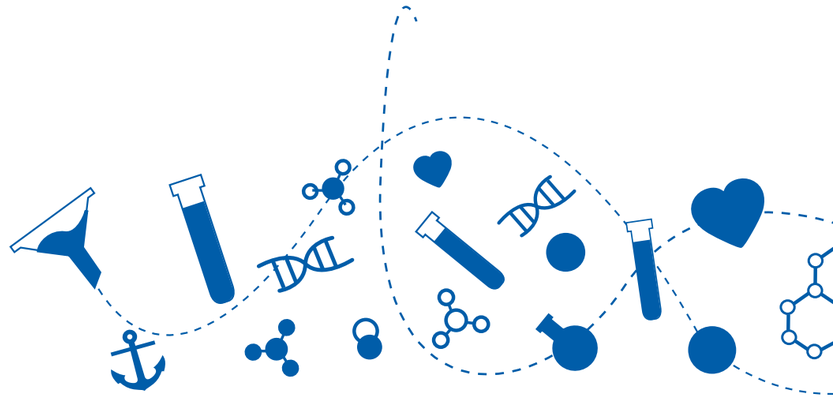

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Custom Products and Services

Our Technologies

Amplite™ enzyme-based detection platform is optimized for measuring horseradish peroxidase (HRP), alkaline phosphates, luciferase, beta-galactosidase, lactamase, oxidase, protein kinases, protein phosphatases, phosphodiesterases, proteases, cytochrome P450, histone deacetylase (HDAC) and cell signaling molecules such as NAD/NADH, NADP/NADPH, IP_3, cAMP and cGMP etc.

Cell Explorer™ cell labeling platform is a complete set of tools for tracking live cells. This platform is also widely used for sorting mixed populations of cells.

Cell Navigator™ cell staining platform is a complete set of tools for selective labeling subcellular structures of live, fixed and dead cells.

Cell Meter™ cellular functional assay platform is a complete set of tools for functional analysis of cellular events and real time-monitoring of cell functions.

iFluor™ superior fluorescent labeling dyes are optimized for labeling proteins and nucleic acids. This group of dyes span from UV to infrared wavelength with good photostability and brightness.

mFluor™ superior fluorescent labeling dyes are optimized for flow cytometry applications.

PhosphoWorks™ detection platform is a set of tools for detection of ATP, ADP, AMP, phosphate, pyrophosphate, phosphoproteins and phosphopeptides.

Quest View™ colorimetric protease platform is a sensitive and robust tool for rapid detection of protease and glycosidase biomarkers. This technology platform has been licensed by a few diagnostic companies for developing rapid diagnostic tests.

RatioWorks™ superior cellular dyes are a sensitive and robust tool set for ratio imaging and real time monitoring of cellular functions (such as pH and ions) in live cells.

Screen Quest™ assay kits are a set of HTS-ready tools for high throughput screening of biochemical and cellular targets such as protein kinases, proteases, HDAC, cell apoptosis and cytotoxicity, GPCR, ion channels, ADME and transporters.

Tide Fluor™ and Tide Quencher™ superior labeling dyes are specially optimized for labeling nucleotides and peptides. This platform offers the best value in the industry. It is second to none in terms of performance and cost. This technology platform has been licensed by a few diagnostic companies for developing IVD diagnostic tests.

trFluor™ superior fluorescent labeling dyes are optimized for developing time-resolved fluorescence-based assays. It has been used for developing HTS assay technologies for many drug discovery targets.

Our Services

Besides the catalog products we also offer custom services to meet the distinct needs of each customer. Our current services include custom synthesis of biological detection probes, custom development of biochemical, cell-based and diagnostic assays, custom bioconjugation and custom high throughput screening of drug discovery targets.

Custom Assay Design and Development

At AAT Bioquest we not only make probes and assay kits, but also use them extensively ourselves. Scientists at AAT Bioquest are experts on assay design and have developed a wide variety of tests that range from biochemical detection to cellular functions. Our assay options include:

- Enzyme activities
- Binding assays
- Cell-based assays
- Microplate assays
- Flow cytometric analysis
- Fluorescence imaging

Custom Conjugation

AAT Bioquest offers the best and the most rapid bioconjugation service in the industry.

- Biotinylation
- Fluorescence labeling (iFluor™, mFluor™, APC, RPE and PerCP)
- Enzyme labeling (AP and HRP)
- Small molecule conjugation

Custom Screening

AAT Bioquest offers on-demand high-throughput screening and pharmacology profiling assays with multiple methodologies. Functional assays are designed, validated and customized to the needs of our pharmaceutical and biotechnology industry clients. These assays are aimed at assessing and monitoring the efficacy, tolerability and safety parameters of candidate compounds for treating and/or diagnosing cancer, infectious disease, autoimmunity and transplantation. Our screening options include:

- Full assay development for a target of your choice
- Optimization of your assay protocol for HTS
- Multiple assay platforms and detection methods
- Custom data analysis

Custom Synthesis of Fluorophores and Luminophores

AAT Bioquest is recognized by the top pharmaceutical companies and diagnostic companies as a key provider of novel fluorescent dyes and luminescent probes. Over the years we have developed and synthesized many enabling fluorescent and luminescent probes for running a variety of challenging biological detection tasks.

Apoptosis Assays and Probes

Apoptosis is the process of programmed cell death that may occur in multicellular organisms. Apoptotic biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis generally confers advantages during an organism's life cycle. Unlike necrosis, apoptosis produces cell fragments called apoptotic bodies that phagocytic cells are able to engulf and quickly remove before the contents of the cell can spill out onto surrounding cells and cause damage. The balance of cell proliferation and apoptosis is important for both development and normal tissue homeostasis. Cell proliferation is an increase in the number of cells as a result of growth and division. Cell proliferation is regulated by the cell cycle, which is divided into a series of phases. Apoptosis, or programmed cell death, results in controlled self-destruction.

Research in and around apoptosis has increased substantially since the early 1990s. In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in an extensive variety of diseases. Excessive apoptosis causes atrophy, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer. AAT Bioquest carries a comprehensive portfolio of reagents for the study of apoptosis, cell cycle, and cell proliferation in a variety of samples.

2.1 Apoptosis-Induced Changes in Cytoplasm

Caspase Activity Assays

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

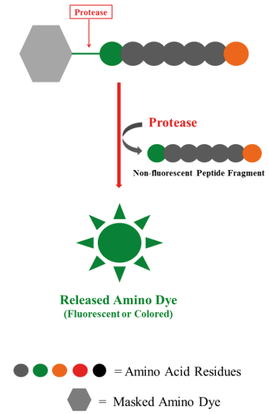

Figure 2.1. The caspase-sensitive peptide fragment-masked amino dyes are digested by a caspase to generate the highly fluorescent dye (or a highly colored dye). The fluorescence (or color) intensity increase is proportional to the caspase activity.

AAT Bioquest offers a diverse selection of capase inhibitors, chromogenic and fluorogenic caspase substrates, and caspase assay kits. Our chromogenic caspase substrates are based on 4-nitroaniline (4-PNA). AAT Bioquest is the only company that offers the multicolor substrates of four distinct fluorescence colors based on 7-Amino-4-methylcoumarin (AMC), 7-Amino-4-trifluoromethyl-coumarin (AFC), Rhodamine 110 (R110) and ProRed™ respectively (see Figure 2.2). In particular, the ProRed™-based caspase substrates are extremely useful for screening caspase inhibitors due to their longer excitation and emission wavelengths that eliminate the autofluorescence interference from the compound library.

Table 2.1 Features of Different Apoptosis Probes

Parameters Measured	Probes	Key Features
Plasma Membrane Alterations (PS Exposure)	Annexin Binding Assay	Detect early apoptosis markers Flow cytometry or immunofluorescence application
Caspase Activation (Cytoplasm)	Caspase Activity Assay	Quick, easy and high throughput
Caspase Binding (Cytoplasm)	Fluorescent Caspase Inhibitors	ELISA, flow cytometry, or Western blot
DNA Fragmentation (Nucleus)	BrdU Assay TUNEL Assay	Work with adherent cells, conjugated single cell resolution with cell cycle analysis by flow cytometry
Mitochondrial Changes	Mitochondrial Stains	Fast, easy, single cell resolution using flow cytometry, fluorescence microscopy, or fluorescence microplate readers

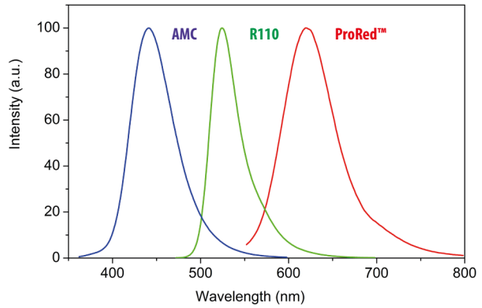

Figure 2.2. The normalized fluoresence spectra of AMC, R110 and ProRed™ in aqueous buffer (pH 7.0). AMC, R110 and ProRed™ caspase substrates are well suited for multiplexing caspase activities.

Caspase 3/7 Detection

Caspase 3 (CPP32/apopain) is a key effector in the apoptosis pathway, amplifying the signal from initiator caspases (such as caspase 8) and signifying full commitment to cellular disassembly. In addition to cleaving other caspases in the enzyme cascade, caspase 3 has been shown to cleave poly(ADP-ribose) polymerase (PARP), DNA-dependent protein kinase, protein kinase C_δ and actin.

DEVD peptide sequence is selective for caspases 3/7. It has been used to develop a number of caspase 3/7 substrates. The Z-DEVD-R110 substrate is a nonfluorescent bisamide that is first converted by caspase 3/7 (or a closely related protease) to the fluorescent monoamide and then to the even more fluorescent R110 (excitation/emission maxima ~496/520 nm). R110-based caspase substrates are more sensitive than coumarin-based caspases substrates (e.g., AMC and AFC), but have narrower dynamic ranges due to the two-step cleavage process. We recommend that R110-based caspase substrates are used for end point assays while AMC and AFC caspase substrates are used for kinetic assays. Our ProRed™-DEVD substrates are extremely useful for screening caspase 3/7 inhibitors due to its longer excitation and emission wavelengths.

Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit (Cat# 22797) is designed to monitor cell apoptosis through measuring caspase 3 activation. Caspase 3 is widely accepted as a reliable indicator for cell apoptosis since the activation of caspase 3 is important for the initiation of apoptosis. Caspase 3 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). Z-DEVD-ProRed™ is used in the kit as the fluorogenic indicator for caspase 3 activity. Cleavage of ProRed™ DEVD blocking peptide residue by caspase 3 generates strongly red fluorescent ProRed™ that is fluorimetrically monitored at ~620 nm with excitation at ~530 nm. Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit is robust and can be readily adapted for high throughput assays in a wide variety of fluorescence platforms such as microplate assays. Using 25 µL of reagents per well in a 384-well format, the kit provides sufficient reagents to perform 400 assays.

Table 2.2 Caspase Activity Assay Reagents

Cat. #	Product Name	Biological Function	Size	Ex (nm)	Em (nm)
13401	Ac-DEVD-AFC	Fluorogenic Caspase 3/7 Substrate	5 mg	380	500
13402	Ac-DEVD-AMC	Fluorogenic Caspase 3/7 Substrate	5 mg	351	430
13403	Ac-DEVD-CHO	Caspase 3/7 Inhibitor	1 mg	N/A	N/A
13405	Ac-DEVD-pNA	Chromogenic Caspase 3/7 Substrate	5 mg	408	N/A
13410	Ac-IETD-AFC	Fluorogenic Caspase 8 Substrate	5 mg	380	500
13411	Ac-IETD-AMC	Fluorogenic Caspase 8 Substrate	5 mg	351	430
13412	Ac-IETD-CHO	Caspase 8 Inhibitor	5 mg	N/A	N/A
13431	$(Ac\text{-}IETD)_2$-R110	Fluorogenic Caspase 8 Substrate	1 mg	498	520
13426	Ac-LEHD-AMC	Fluorogenic Caspase 9 Substrate	5 mg	351	430
13427	$(Ac\text{-}LEHD)_2$-R110	Fluorogenic Caspase 9 Substrate	1 mg	498	520
13420	Z-DEVD-AFC	Fluorogenic Caspase 3/7 Substrate	5 mg	380	500
13421	Z-DEVD-AMC	Fluorogenic Caspase 3/7 Substrate	5 mg	351	430
13422	Z-DEVD-pNA	Chromogenic Caspase 3/7 Substrate	5 mg	408	N/A
13425	Z-IETD-AFC	Fluorogenic Caspase 8 Substrate	5 mg	380	500
13413	Z-IETD-pNA	Chromogenic Caspase 8 Substrate	5 mg	408	N/A
13433	Z-DEVD-ProRed™ 620	Fluorogenic Caspase 3/7 Substrate	1 mg	534	619
13430	$(Z\text{-}DEVD)_2$-R110	Fluorogenic Caspase 3/7 Substrate	1 mg	498	520
13434	Z-IETD-ProRed™ 620	Fluorogenic Caspase 8 Substrate	1 mg	534	619
13435	Z-LEHD-ProRed™ 620	Fluorogenic Caspase 9 Substrate	1 mg	534	619

Apoptosis in Cytoplasm

2

Apoptosis Assays and Probes

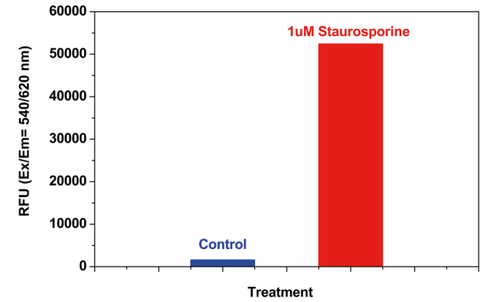

Figure 2.3. Detection of caspase 3/7 activities with Kit 13504. Jurkat cells were seeded on the same day at 200,000 cells /well/90 µL. The fluorescence intensity was measured at Ex/Em = 540/620 nm.

Table 2.3 Caspase 3/7 Activity Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
13502	Amplite™ Fluorimetric Caspase 3/7 Assay Kit *Blue Fluorescence*	500 tests	351	430
13503	Amplite™ Fluorimetric Caspase 3/7 Assay Kit *Green Fluorescence*	500 tests	498	520
13504	Amplite™ Fluorimetric Caspase 3/7 Assay Kit *Red Fluorescence*	100 tests	534	619
22795	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 tests	351	430
22796	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Green Fluorescence*	200 tests	498	520
22797	Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Red Fluorescence*	100 tests	534	619

Caspase 8 Detection

Caspase 8 plays a critical role in the early cascade of apoptosis, acting as an initiator of the caspase activation cascade. Activation of the enzyme itself is accomplished through direct interaction with the death domains of cell-surface receptors for apoptosis-inducing ligands. The activated protease has been shown to be involved in a pathway that mediates the release of cytochrome c from the mitochondria, and is also known to activate downstream caspases, such as caspase 3. IETD peptide sequence is selective for caspases 8. AAT Bioquest offers both caspase reagents and assay kits for detecting caspase 8 (see Tables 2.2 and 2.4).

Table 2.4 Caspase 8 Activity Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22812	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 tests	351	430
22798	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Green Fluorescence*	200 tests	498	520
22816	Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Red Fluorescence*	100 tests	534	619

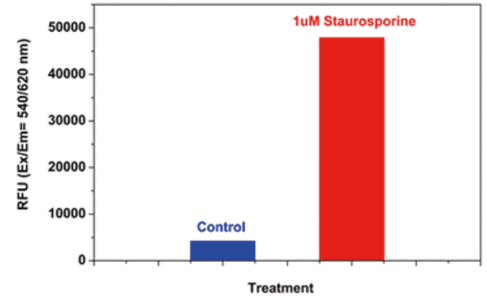

Figure 2.4. Detection of caspase 8 activities with Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit (Cat# 22816). Jurkat cells were seeded on the same day at 200,000 cells/well/90 µL. The cells were treated with staurosporine at the final concentration of 1 µM for 5 hours while the untreated cells were used as control. The caspase 8 assay solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 540/620 nm with FlexStation fluorescence microplate reader (Molecular Devices).

Caspase 9 Detection

Caspase 9 is a member of the CED-3 subfamily of the caspase family of cysteine proteases that play an essential role in the execution phase of apoptosis. LEHD peptide sequence is selective for caspase 9. AAT Bioquest offers PNA, AMC, AFC, R110 and ProRed™ caspase 9 substrates that contain the LEHD peptide fragment for caspase 9 selectivity (See Table 2.2 and 2.5).

Cell Meter™ Caspase 9 Activity Apoptosis Assay Kits are designed to monitor cell apoptosis by measuring caspase 9 activity. Kit #22799 uses $(Ac\text{-}LEHD)_2$-R110 as a fluorogenic indicator for caspase 9 activity while Kit #22813 uses Ac-LEHD-AMC to monitor caspase 9 activity. Cleavage of R110 peptides by caspase 9 generates strongly green fluorescent R110 while Ac-LEHD-AMC produces strongly blue fluorescent AMC upon interacting with caspase 9. Both of the kits provide all the essential components. The assays are robust and can be readily adapted for high throughput screening. It can be used to either quantify the activated caspase 9 activities in apoptotic cells or screen caspase 9 inhibitors.

Table 2.5 Caspase 9 Activity Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22813	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Blue Fluorescence*	200 tests	351	430
22799	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Green Fluorescence*	200 tests	498	520
22817	Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Red Fluorescence*	100 tests	534	619
22820	Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit *Tricolor Fluorescence*	3x100 tests	Multiple Colors	

For Pricing Information Please Visit: **www.biomol.de**

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Not for use in diagnostic or therapeutic procedures.

Multiplexing Detection of Caspases 3, 7, 8 and 9

AAT Bioquest has developed Cell Meter™ Caspase 3/7, 8 and 9 Activity Multiplexing Assay Kit (Cat# 22820) for multiplexing the detection of caspases 3, 7, 8 and 9. This particular kit is designed to simultaneously monitor four key caspases (caspase 3/7, 8 and 9) activation involved in cell apoptosis using the three distinct fluorescent colors. This kit uses DEVD-ProRed™, IETD-R110 and LEHD-AMC as fluorogenic indicators for caspase 3/7, 8 and 9 activity respectively. Upon caspase cleavages, DEVD-ProRed™, IETD-R110 and LEHD-AMC caspase substrates generate three distinct fluorophores: ProRed™ (red fluorescence), R110 (green fluorescence) and AMC (blue fluorescence), which can be readily monitored in a single assay due to their nice spectral separation (see Figure 2.2).

Table 2.6 Multiplexing Caspase Activity and Apoptosis Assay Kits

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

Caspase Binding Assays

In the process of apoptosis, one of the key events is the activation of caspases, which is important for the initiation of apoptosis. Cell Meter™ Live Cell Caspase Binding Kits use fluorescent cell permeable and nontoxic indicators to detect caspase 1, 2, 3/7, 6, 8, 9, 10, and 13 activities. Once bound to caspases, the fluorescent reagents are retained inside the cell. The binding event prevents the caspases from further catalysis but will not stop apoptosis from proceeding. The caspase binding kits are applicable for fluorescence microscope, flow cytometer, and fluorescence microplate reader. The kits provide all the essential components with an optimized assay protocol.

Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit (Cat# 22850) is designed to detect apoptosis by simultaneously monitoring Caspase 3/7 and Annexin V activities in mammalian cells. Annexins are a family of proteins that bind to phospholipid membranes in the presence of calcium. Annexin V is used to detect apoptotic cells that express phosphatidylserine (PS) on the cell surface. The appearance of PS on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis. Annexin V-dye conjugates monitor cell apoptosis through measuring the translocation of PS. The kit also provides a Hoechst dye for labeling the nucleus of the whole population of the cells, and propidium iodide dye for staining necrosis cells.

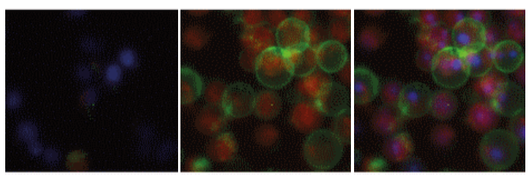

Figure 2.5. The detection of caspase binding activities with Kits 20101 and 22791. The fluorescence image analysis indicated the increased expression of caspase 3/7 (red, stained by TF3-DEVD-FMK) and Annexin V (green, stained by Annexin V-iFluor™ 488) in Jurkat cells induced by 1 µM staurosporine for 3 hours. The fluorescence images of the cells (300,000 cells/ well) were taken with Olympus fluorescence microscope using the DAPI, FITC, and TRITC channel respectively. Individual images of the same cell population were merged as shown above. A: Non-induced control cells; B: Double staining of staurosporine-induced cells for caspase 3/7 (red) and Annexin V (green); C. Triple staining of staurosporine-induced cells for caspase 3/7 (red), Annexin V (green) and nucleus (blue).

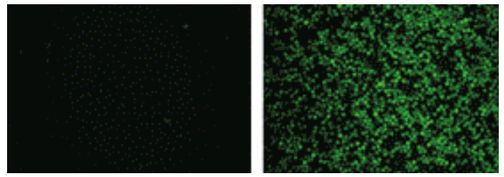

Figure 2.6. The fluorescence imaging demonstrated the increase in FITC-C6-DEVD-FMK (Cat# 13408) fluorescence intensity with the addition of 1 µM staurosporine in Jurkat cells. Cells were incubated with FITC-C6-DEVD-FMK for 1 hour at 37 ºC. The fluorescence intensity of the cells (200,000 cells/100 µL/well) was viewed under a fluorescence microscope using the FITC channel. A: Control; B: Staurosporine-treated.

Table 2.7 Caspase Binding Based Live Cell Apoptosis Reagents and Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
20108	Cell Meter™ Live Cell Caspase 1 Binding Assay Kit	25 tests	492	514
20111	Cell Meter™ Live Cell Caspase 2 Binding Assay Kit	25 tests	492	514
22850	Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit	100 tests	Multiple Colors	
20100	Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit *Green Fluorescence*	25 tests	492	514
20101	Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit *Red Fluorescence*	25 tests	556	574
20113	Cell Meter™ Live Cell Caspase 6 Binding Assay Kit	25 tests	492	514
20115	Cell Meter™ Live Cell Caspase 8 Binding Assay Kit	25 tests	492	514
20117	Cell Meter™ Live Cell Caspase 9 Binding Assay Kit	25 tests	492	514
20119	Cell Meter™ Live Cell Caspase 10 Binding Assay Kit	25 tests	492	514
20125	Cell Meter™ Live Cell Caspase 13 Binding Assay Kit	25 tests	492	514
13470	FAM-VAD-FMK	25 tests	492	518
13408	FITC-C6-DEVD-FMK	100 µg	492	516
13409	FITC-C6-LEHD-FMK	100 µg	492	516
13475	mFluor™ 450-VAD-FMK	25 tests	403	454
13476	mFluor™ 510-VAD-FMK	25 tests	414	508
13472	SRB-VAD-FMK [Sulforhodamine B-VAD-FMK]	25 tests	556	575
13471	TF4-VAD-FMK	25 tests	588	610
13420	Z-DEVD-AFC	5 mg	380	500
13421	Z-DEVD-AMC	5 mg	351	430
13433	Z-DEVD-ProRed™ 620	1 mg	534	619
13435	Z-IEHD-ProRed™ 620	1 mg	534	619
13425	Z-IETD-AFC	5 mg	380	500
13434	Z-IETD-ProRed™ 620	1 mg	534	619

GSH-Monitoring Apoptosis Assays

There are a variety of parameters that can be used for monitoring cell apoptosis. This particular kit is designed to detect cell apoptosis by measuring the decrease in reduced glutathione (GSH). GSH is important for maintaining redox level of cells. It is involved in many cellular processes including the scavenging of free radicals, drug detoxification, cell signaling, and cell proliferation. The decrease in cellular GSH concentration is an early hallmark in the progression of cell death in response to different apoptotic stimuli in many cells.

Cell Meter™ Intracellular GSH Assay Kit (Cat# 22810) uses our proprietary non-fluorescent Thiolite™ Green, which becomes strongly fluorescent upon reacting with thiol (including GSH in cells). In normal cells, Thiolite™ Green is accumulated primarily in cytosol, but it is partially translocated to mitochondria in apoptotic cells while Thiolite™ Green staining intensity is decreased. Cells stained with Thiolite™ Green can be visualized with flow cytometer at Ex/Em = 490/520 nm (FL1 channel). The kit can be used together with other reagents, such as 7-AAD (Cat#17501) for multi-parametric study of cell viability and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors with flow cytometer.

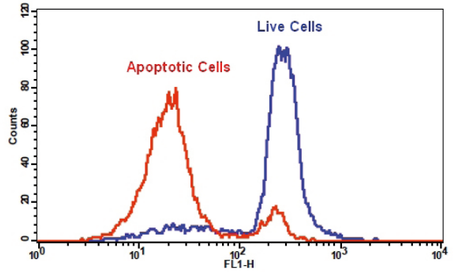

Figure 2.7. The detection of GSH in apoptotic cells with Kit 22810. The decrease in the fluorescence intensity of Thiolite™ Green adduct with the addition of camptothecin in Jurkat cells. Jurkat cells were treated overnight without (blue) or with 20 µM camptothecin (red) in a 37 ºC, 5% CO_2 incubator, and loaded with Thiolite™ Green for 30 minutes. The fluorescence intensity of Thiolite™ Green was measured with a FACSCalibur flow cytometer using FL1 channel.

Table 2.8 Intracellular GSH Assay Kits and Probes

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
5524	Amplite™ Fluorimetric Thiol Quantitation Assay Kit *Green Fluorescence*	1 kit	490	515
22810	Cell Meter™ Intracellular GSH Assay Kit *Optimized for Flow Cytometry*	1 kit	490	515
21507	Thiolite™ Blue	5 mg	335	460
21506	Thiolite™ Blue, AM	1 mg	335	460

2.2 Apoptosis-Induced Changes in Plasma Membrane

Phosphatidylserine Binding Assays Using Annexin V Conjugates

The apoptotic process is characterized by certain morphological features. The features include changes in the plasma membrane (such as loss of membrane symmetry and loss of membrane attachment), a condensation of the cytoplasm and nucleus, protein cleavage, and internucleosomal cleavage of DNA. In the final stages of the process, dying cells become fragmented into "apoptotic bodies" and consequently eliminated by phagocytic cells without significant inflammatory damage to surrounding cells.

Changes in the plasma membrane are one of the first characteristics of the apoptotic process detected in living cells. Apoptosis can be detected by the presence of phosphatidylserine (PS), which is normally located on the cytoplasmic face of the plasma membrane. During apoptosis, phosphatidylserine translocates to the outer leaflet of the plasma membrane and can be detected by flow cytometry and cell imaging through binding to fluorochrome-labeled Annexin V conjugates when calcium is present.

Annexins are a family of calcium-dependent phospholipid-binding proteins. They are abundant in eukaryotic organisms belonging to a family of ubiquitous cytoplasmic proteins involved in signal transduction. Annexin V's preferential binding partner is phosphatidylserine, which is usually kept on the inner-leaflet (the cytosolic side) of cell membranes. In apoptosis, phosphatidylserine is transferred to the outer leaflet of the plasma membrane. The appearance of phosphatidylserine on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis and can be detected before morphological changes can be observed.

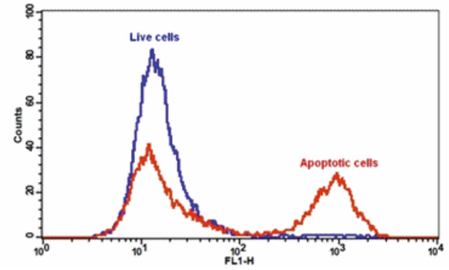

Figure 2.8. The detection of binding activity of Annexin V-iFluor™ 488 (Cat# 20071) to phosphatidylserine in Jurkat cells. Jurkat cells were treated without (blue) or with 20 µM camptothecin (red) in a 37 ºC, 5% CO_2 incubator for 4-5 hours, and then dye loaded with Annexin V-iFluor™ 488 for 30 minutes. The fluorescence intensity of Annexin V-iFluor™ 488 was measured with a FACSCalibur™ flow cytometer using the FL1 channel.

Cell Meter™ Annexin V Binding Apoptosis Assay Kits use our proprietary fluorescent Annexin V-iFluor™ PS sensors that specifically bind PS with good photostability. The kits provide all the essential components with an optimized protocol. Cell Meter™ Phosphatidylserine Apoptosis Assay Kits use Apopxin™ PS sensors. Due to the highly enhanced affinity to phosphatidylserine, the kits are more robust than other commercial Annexin V-based apoptosis kits that are only used with either microscope or flow cytometry platform. The kits can be used with a fluorescence microplate reader besides the microscope and flow cytometry platforms. They have been used for HTS applications.

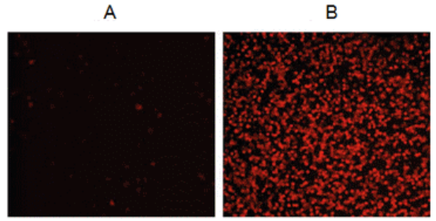

Figure 2.9. Images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with the Cell Meter™ AnnexinV Binding Apoptosis Assay Kit *Red Fluorescence* (Cat# 22826). A: Untreated control cells B: Cells treated with 1 µM staurosporine for 5 hours.

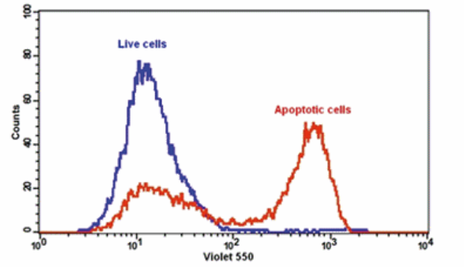

Figure 2.10. The detection of binding activity with Cell Meter™ Annexin V Binding Apoptosis Assay Kit (Cat# 22830) in Jurkat cells. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO_2 incubator for 5 hours, and then dye loaded with Annexin V-mFluor Violet™ 540 for 30 minutes. The fluorescence intensity of Annexin V-mFluor Violet™ 540 was measured with a FACSCalibur™ (Becton Dickinson, San Jose, CA) flow cytometer using violet laser at Ex/Em = 405/550 nm.

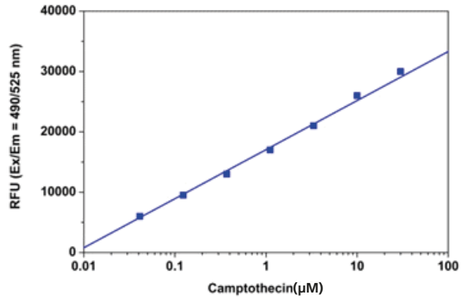

Figure 2.11. The detection of phosphatidylserine binding activity in Jurkat cells with Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22791). Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with different doses of camptothecin for 5 hours as indicated. The Apopxin™ Green assay solution (100 µL/well) was added & incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 490/525 nm with NOVOstar instrument using bottom read mode. Our Cell Meter™ Phosphatidylserine Apoptosis Assay Kits are the only commercial products available for monitoring phosphatidylserine binding in live cells using a microplate reader.

Annexin V may be conjugated to fluorochromes including APC and PE (Cat# 22837 & 22838). This format retains its high affinity for phosphatidylserine (PS) and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis. Since externalization of PS occurs in the earlier stages of apoptosis, APC/PE Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation. APC/PE Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with APC/PE Annexin V is typically used in conjunction with a vital dye such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (7-AAD negative, APC Annexin V positive). Viable cells with intact membranes exclude 7-AAD, whereas the membranes of dead and damaged cells are permeable to 7-AAD. For example, cells that are considered viable are both APC/PE Annexin V and 7-AAD negative while cells that are in early apoptosis are APC Annexin V positive and 7-AAD negative, while cells that are in late apoptosis or already dead are both APC/PE Annexin V and 7-AAD positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both APC/PE Annexin V and 7-AAD. However, when apoptosis is measured over time, cells can be often tracked from APC/PE Annexin V and 7-AAD negative (viable, or no measurable apoptosis), to APC/PE Annexin V positive and 7-AAD negative (early apoptosis, membrane integrity is present) and finally to APC/PE Annexin V and 7-AAD positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both APC/PE Annexin V and 7-AAD positive, in of itself, reveals less information about the process by which the cells underwent their demise.

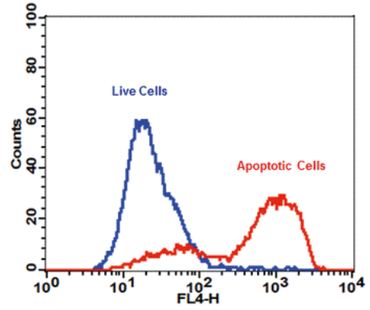

Figure 2.12. The detection of binding activity with Cell Meter™ APC-Annexin V Binding Apoptosis Assay Kit (Cat# 22837) in Jurkat cells. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO_2 incubator for ~4 hours, and then dye loaded with APC-Annexin V for 30 minutes. The fluorescence intensity of APC-Annexin V was measured with a FACSCalibur™ (Becton Dickinson, San Jose, CA) flow cytometer using the FL4 channel.

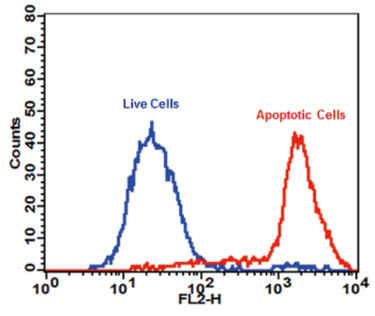

Figure 2.13. The detection of binding activity with Cell Meter™ PE-Annexin V Binding Apoptosis Assay Kit (Cat# 22838) in Jurkat cells. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO_2 incubator for 4-5 hours, and then dye loaded with PE-Annexin V for 30 minutes. The fluorescence intensity of PE-Annexin V was measured with a FACSCalibur™ (Becton Dickinson, San Jose, CA) flow cytometer using the FL2 channel.

Table 2.9 Annexin V Binding Based Cell Apoptosis Reagents and Assay Kits

Cat. #	Product Name	Size
20030	Annexin V, FITC Labeled	100 tests
20031	Annexin V, TRITC Labeled	100 tests
20070	Annexin V-iFluor™ 350 Conjugate	100 tests
20071	Annexin V-iFluor™ 488 Conjugate	100 tests
20072	Annexin V-iFluor™ 555 Conjugate	100 tests
20073	Annexin V-iFluor™ 594 Conjugate	100 tests
20074	Annexin V-iFluor™ 647 Conjugate	100 tests
20075	Annexin V-iFluor™ 680 Conjugate	100 tests
20077	Annexin V-iFluor™ 700 Conjugate	100 tests
20076	Annexin V-iFluor™ 750 Conjugate	100 tests
20085	Annexin V-mFluor™ Blue 570 Conjugate	100 tests
20080	Annexin V-mFluor™ Violet 450 Conjugate	100 tests
20081	Annexin V-mFluor™ Violet 510 Conjugate	100 tests
20082	Annexin V-mFluor™ Violet 540 Conjugate	100 tests
22828	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Blue Fluorescence Excited at 405 nm*	100 tests
22827	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Flow Cytometry*	100 tests
22829	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Green Fluorescence Excited at 405 nm*	100 tests
22824	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests
22830	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Orange Fluorescence Excited at 405 nm*	100 tests
22825	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*	100 tests
22826	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 tests
22837	Cell Meter™ APC-Annexin V Binding Apoptosis Assay Kit *Optimized for Flow Cytometry*	100 tests
22850	Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit *Triple Fluorescence Colors*	100 tests
22838	Cell Meter™ PE-Annexin V Binding Apoptosis Assay Kit *Optimized for Flow Cytometry*	100 tests
22835	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Blue Fluorescence Excited at 405 nm*	100 tests
22790	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Blue Fluorescence Optimized for Microplate Readers*	100 tests
22832	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Flow Cytometry*	100 tests
22793	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Microplate Readers*	100 tests
22836	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Excited at 405 nm*	100 tests
22831	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests
22791	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Optimized for Microplate Readers*	100 tests
22794	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Orange Fluorescence Optimized for Microplate Readers*	100 tests
22792	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Red Fluorescence Optimized for Microplate Readers*	100 tests

2.3 Apoptosis-Induced Changes in Mitochondria

Mitochondrial dysfunction has been shown to participate in the induction of apoptosis and has even been suggested to be central to the apoptotic pathway. Opening of the mitochondrial permeability transition pore has been demonstrated to induce depolarization of the transmembrane potential, release of apoptogenic factors and loss of oxidative phosphorylation. In some apoptotic systems, loss of mitochondrial membrane potential (MMP) may be an early event in the apoptotic process. During apoptosis, the mitochondrial membrane potential (MMP) decreases, thus MMP change has been used for monitoring cell apoptosis. Early after growth factor withdrawal the MMP declines and the matrix condenses. There are quite a few fluorescent dyes (such as JC-1 and JC-10™) that are increasingly used for monitoring cell apoptosis through tracking MMP.

JC-1 and JC-10™

JC-1 exists as a green-fluorescent monomer at low concentrations or at low membrane potentials. However, at higher concentrations (aqueous solutions above 0.1 µM) or higher potentials, JC-1 forms red-fluorescent "J-aggregates," which 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 $DiOC_6(3)$ 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), 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™ to be a 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 entering selectively 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. For the first time, we have developed a protocol to use JC-10™ in fluorescence microplate platform. In some cell lines JC-10™ has far superior performance to JC-1.

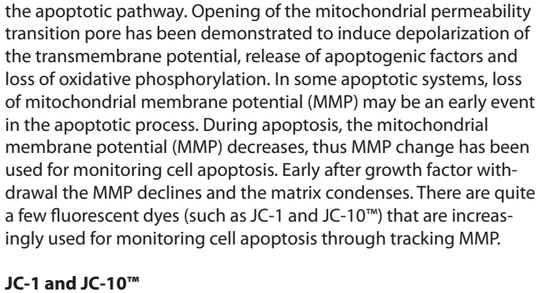

Figure 2.14. The detection of FCCP-induced mitochondrion membrane potential changes with Cell Meter™ JC-10™ Mitochondrion Membrane Potential Assay Kit *Optimized for Flow Cytometry Assays* (Cat# 22801) in Jurkat cells. Jurkat cells were dye loaded with JC-10™ dye-loading solution along with DMSO (left) or 20 µM FCCP (right) for 10 minutes. The fluorescence intensities for both J-aggregates and monomeric forms of JC-10™ were measured with FACSCalibur™ flow cytometer (Becton Dickinson) using FL1 and FL2 channels after compensation.

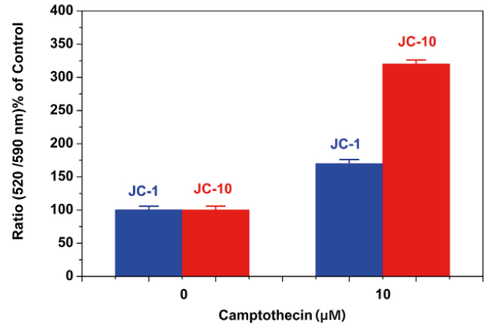

Figure 2.15. Camptothecin induced mitochondrion membrane potential changes were measured with JC-10™ (Cat# 22204) and JC-1 (Cat# 22200) in Jurkat cells. After Jurkat cells were treated with camptothecin (10 µM) for 4 hours, JC-1 and JC-10™ dye loading solutions were added to the wells and incubated for 30 minutes. The fluorescence intensities for both J-aggregates and monomeric forms of JC-1 and JC-10™ were measured at Ex/Em = 490/525 nm and 540/590 nm with NOVOstar microplate reader (BMG Labtech).

Mitochondrion Membrane Potential Assay Kits

Cell Meter™ Mitochondrion Membrane Potential (MMP) Assay Kits provide all the essential components with an optimized assay method. These fluorimetric assays use our proprietary cationic mitochondrial probes for the detection of mitochondrial membrane potential changes. In normal cells, the red fluorescence intensity is increased when mitochondrial probes are accumulated in the mitochondria. However, in apoptotic cells, the fluorescence intensity of mitochondrial dyes is decreased following the collapse of MMP. The collapse of mitochondrial membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol, which in turn triggers other downstream events in the apoptotic cascade. These kits are individually optimized for screening apoptosis activators and inhibitors with a flow cytometer, a fluorescence microscope or a fluorescence microplate reader.

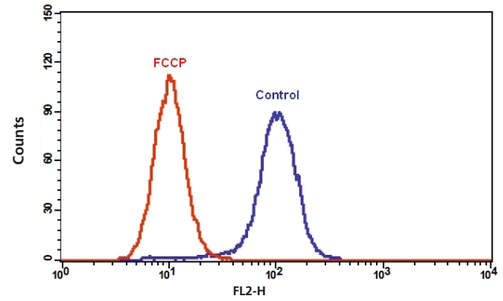

Figure 2.16. The detection of FCCP-induced mitochondrion membrane potential change with Cell Meter™ Mitochondrion Membrane Potential Assay Kit *Orange Fluorescence Optimized for Flow Cytometry* (Cat# 22804). The decrease in fluorescence intensity of MitoTell™ Orange with the addition of FCCP in Jurkat 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.

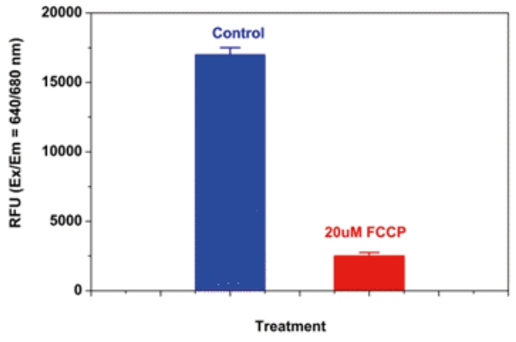

Figure 2.17. The detection of FCCP-induced mitochondrion membrane potential change with Cell Meter™ NIR Mitochondrion Membrane Potential Assay Kit *Optimized for Microplate Reader* (Cat# 22803). HeLa cells were dye loaded with MitoLite™ NIR alone or in the presence of 20 µM FCCP for 15 minutes. The fluorescence intensity of MitoTell™ NIR was measured 30 minutes after adding Assay Buffer B (Component C) with a FlexStation™ microplate reader (Molecular Devices) at Ex/Em = 640/680 nm (cut off 665 nm, bottom read).

2.4 Mitochondrion Staining Probes

Cell Navigator™ Mitochondrion Staining Kits are designed to label mitochondria of live cells with a full set of fluorescence colors including blue, green, orange and NIR fluorescence. The kits use proprietary dyes that selectively accumulate in mitochondria probably via the mitochondrial membrane potential gradient. The mitochondrial indicators are retained in mitochondria for a long time and show good photostability. This key feature significantly increases the staining efficiency. The labeling protocol is robust, requiring minimal hands-on time. It can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

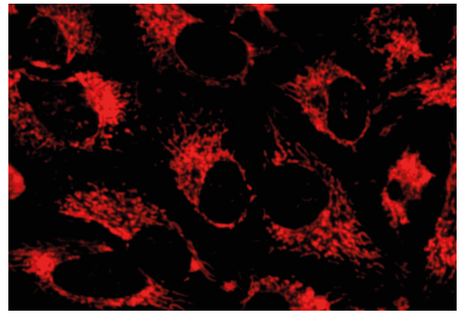

Figure 2.18. Image of HeLa cells stained with Cell Navigator™ Mitochondrion Staining Kit *Red Fluorescence* (Cat# 22668) in a Costar black wall/clear bottom 96-well plate. The filter set of Texas Red® was used for imaging.

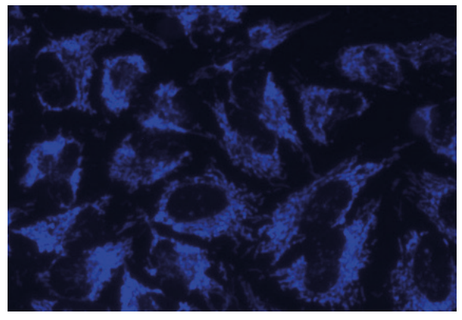

Figure 2.19. Image of HeLa cells stained with the Cell Navigator™ Mitochondrial Staining Kit *Blue Fluorescence* (Cat# 22665) in a 96-well clear-bottom plate.

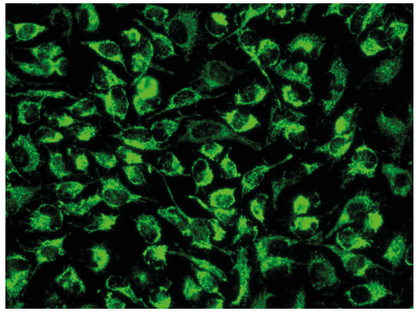

Figure 2.20. Image of U2OS cells stained with the Cell Navigator™ Mitochondrial Staining Kit *Green Fluorescence* (Cat# 22666) in a Costar black 96-well plate.

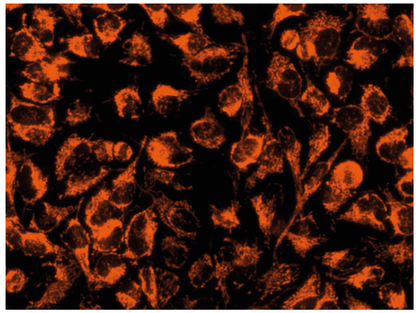

Figure 2.21. Image of U2OS cells stained with the Cell Navigator™ Mitochondrial Staining Kit *Orange Fluorescence with 405 nm Excitation* (Cat# 22673) in a Costar black 96-well plate.

Table 2.10 Mitochondrion Probes and Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22801	Cell Meter™ JC-10™ Mitochondrion Membrane Potential Assay Kit *Optimized for Flow Cytometry Assays*	100 tests	490	525/590
22800	Cell Meter™ JC-10™ Mitochondrion Membrane Potential Assay Kit *Optimized for Microplate Assays*	500 tests	490/540	525/590
22804	Cell Meter™ Mitochondrion Membrane Potential Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*	100 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*	100 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 tests	350	490
22666	Cell Navigator™ Mitochondrion Staining Kit *Green Fluorescence*	500 tests	498	520
22669	Cell Navigator™ Mitochondrion Staining Kit *NIR Fluorescence*	500 tests	640	659
22667	Cell Navigator™ Mitochondrion Staining Kit *Orange Fluorescence*	500 tests	545	575
22673	Cell Navigator™ Mitochondrion Staining Kit *Orange Fluorescence with 405 nm Excitation*	500 tests	399	550
22668	Cell Navigator™ Mitochondrion Staining Kit *Red Fluorescence*	500 tests	575	600
22200	JC-1	5 mg	515	529
22204	JC-10™	5x100 µL	510	525
22674	MitoLite™ Blue FX490	500 tests	350	490
22675	MitoLite™ Green EX488	500 tests	498	520
22678	MitoLite™ NIR 660	500 tests	640	659
22679	MitoLite™ Orange EX405	500 tests	399	550
22676	MitoLite™ Orange FX570	500 tests	640	659
22677	MitoLite™ Red FX600	500 tests	575	600
22210	Rhodamine 123	25 mg	507	529
22220	TMRE	25 mg	549	574
22221	TMRM	25 mg	549	573

2.5 TUNEL Apoptosis Assay

DNA fragmentation represents a characteristic of late stage apoptosis. DNA fragmentation in apoptotic cells can be detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL). The TUNEL assay relies on the presence of nicks in the DNA which can be identified by TdT, an enzyme that catalyzes the addition of dUTPs that are secondarily labeled with a marker. All the existing TUNEL assays contain the highly toxic sodium cacodylate which might induce apoptosis and also decrease DNA production and DNA strands.

Cell Meter™ TUNEL Apoptosis Assay Kit (Cat# 22844) uses proprietary buffer system that is free of sodium cacodylate. The kit is based on incorporation of a fluorescence dye TF3 modified deoxyuridine 5'-triphosphates (TF3-dUTP) at the 3' OH ends of the DNA fragments formed during apoptosis. The assay is optimized for the direct detection of apoptosis in either detached or attached cells without using antibody. The kit provides all the essential components with an optimized assay protocol. It is suitable for fluorescence microplate reader, fluorescence microscope, or flow cytometer. Its signal can be easily detected at Ex/Em = 550/590 nm.

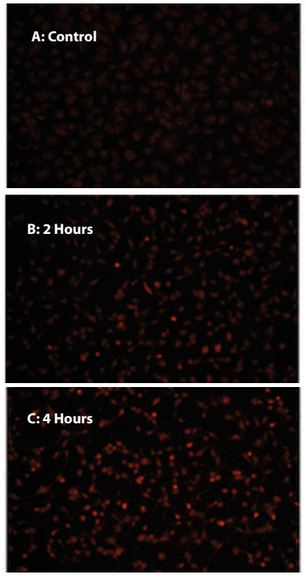

Figure 2.22. The analysis of nuclear apoptosis with Cell Meter™ TUNEL Apoptosis Assay Kit (Cat# 22844). The fluorescence imaging demonstrated the increase in TUNEL reaction with the addition of 1 µM staurosporine for 2 hours (B) or 4 hours (C) compared to control (A) in HeLa cells. Cells were incubated with reaction mixture for 1 hour at 37 ºC. The fluorescence intensity of the cells (30,000 cells/100 µL/well) was analyzed under a fluorescence microscope using the TRITC channel. DNA strand breaks were shown as more intense fluorescent staining spots in cells treated with staurosporine.

Table 2.11 TUNEL Apoptosis Assay Kit

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22844	Cell Meter™ TUNEL Apoptosis Assay Kit	50 tests	556	579

2.6 Necrosis Assays

Apoptosis is an active, programmed process of autonomous cellular dismantling that avoids eliciting inflammation. In apoptosis, phosphatidylserine (PS) is transferred to the outer leaflet of the plasma membrane. As a universal indicator of the initial/intermediate stages of cell apoptosis, the appearance of phosphatidylserine on the cell surface can be detected before morphological changes are observed. Necrosis has been characterized as passive, accidental cell death resulting from environmental perturbations with uncontrolled release of inflammatory cellular contents. Loss of plasma membrane integrity, as demonstrated by the ability of a membrane-impermeable dyes to label the nucleus, represents a straightforward approach to demonstrate late stage apoptosis and necrosis.

Necrosis occurs when cells are exposed to extreme variance from physiological conditions (e.g., hypothermia, hypoxia) which may result in damage to the plasma membrane. Under physiological conditions direct damage to the plasma membrane is evoked by agents like complement and lytic viruses.

Necrosis begins with an impairment of the cell's ability to maintain homeostasis, leading to an influx of water and extracellular ions. Intracellular organelles, most notably the mitochondria, and the entire cell swell and rupture (cell lysis). Due to the ultimate breakdown of the plasma membrane, the cytoplasmic contents including lysosomal enzymes are released into the extracellular fluid. Therefore, *in vivo*, necrotic cell death is often associated with extensive tissue damage resulting in an intense inflammatory response.

Apoptosis, in contrast, is a mode of cell death that occurs under normal physiological conditions and the cell is an active participant in its own demise ("cellular suicide"). It is most often found during normal cell turnover and tissue homeostasis, embryogenesis, induction and maintenance of immune tolerance, development of the nervous system and endocrine-dependent tissue atrophy.

Cells undergoing apoptosis show characteristic morphological and biochemical features. These features include chromatin aggregation, nuclear and cytoplasmic condensation, partition of cytoplasm and nucleus into membrane bound-vesicles (apoptotic bodies) which contain ribosomes, morphologically intact mitochondria and nuclear material. *In vivo,* these apoptotic bodies are rapidly recognized and phagocytized by either macrophages or adjacent epithelial cells. Due to this efficient mechanism for the removal of apoptotic cells in vivo no inflammatory response is elicited. In vitro, the apoptotic bodies as well as the remaining cell fragments ultimately swell and finally lyse.

Cell Meter™ Apoptotic and Necrotic Detection kit (Cat# 22840) is designed to simultaneously monitor apoptotic, necrotic and healthy cells. The kit is optimized to simultaneously detect cell apoptosis, necrosis and healthy cells with a flow cytometer or fluorescence microscope. Kit 22843 can be similarly used. Both of the kits used Calcein AM for monitoring healthy cells, Apopxin™ conjugate for apoptotic cells and a DNA dye for necrotic or dead cells.

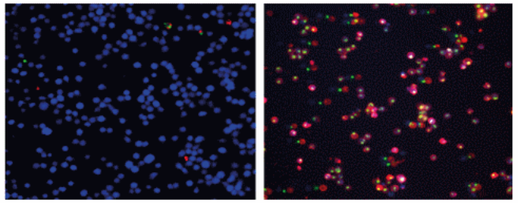

Figure 2.23. The detection of apoptosis and necrosis with Cell Meter™ Apoptotic and Necrotic Detection Kit *Triple Fluorescence Colors* (Cat# 22843). Binding activity of Apopxin™ Deep Red to phosphatidylserine in Jurkat cells. The fluorescence imaging demenstrated that live cells (blue) were stained by CytoCalcein™ Violet 450, apoptotic cells (red) were stained by Apopxin™ Deep Red, and necrotic cells (green) were stained by Nuclear Green™ DCS1. Apoptosis was induced by 1μM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope using the Violet, Cy5® and FITC channel respectively. Individual images taken from each channel from the same cell population were merged as shown above. A: Non-induced control cells; B: Triple staining of staurosporine-induced cells.

Table 2.12 Apoptotic and Necrotic Detection Assay Kits

Cat. #	Product Name	Size
22840	Cell Meter™ Apoptotic and Necrotic Detection Kit *Triple Fluorescence Colors*	100 tests
22843	Cell Meter™ Apoptotic and Necrotic Detection Kit *Triple Fluorescence Colors*	100 tests

2.7 Autophagy Assay

Autophagy is one of the major pathways for degradation of intracellular macromolecules in animal cells. The process of autophagy involves the sequestration of cytoplasmic materials and intracellular organelles in a membrane-bounded vacuole called autophagosome, the fusion of the autophagosome with lysosomes, and the subsequent degradation of sequestered materials.

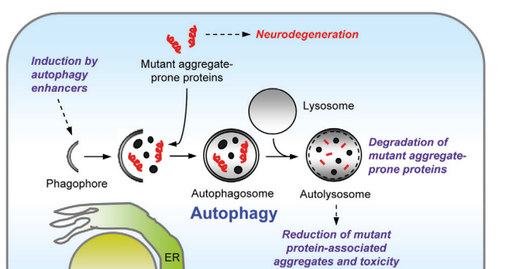

Figure 2.16. The autophagy process.

Cell Meter™ Autophagy Assay Kit (Cat# 23002) and Cell Meter™ Autophagy Fluorescence Imaging Kit (Cat# 23001) employ specific autophagosome markers to analyze the activity of autophagy. The assay is optimized for direct detection of autophagy in both detached and attached cells. The kits provide all the essential components for the assay protocol. They are optimized for fluorescence microscope, and they are also suitable for flow cytometer. Cell Meter™ Autophagy Assay Kit is suitable for microplate reader, too.

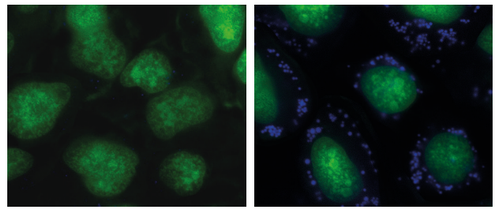

Figure 2.24. Cell Meter™ Autophagy Fluorescence Imaging Kit (Cat# 23001) labeled vesicles were induced by starvation in HeLa cells. HeLa cells were incubated in a regular DMEM medium (Left: Control) or in 1X HBSS buffer with 5% serum (Right: Autophagy Treatment) for 16 hours. Both control cells and treated 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 (Cat#17540, green).

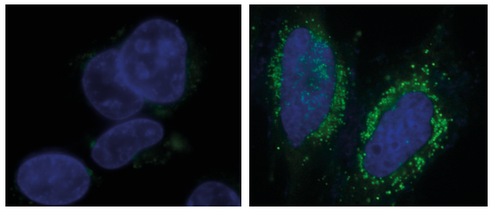

Figure 2.25. Cell Meter™ Autophagy Assay Kit *Green Fluorescence* (Cat# 23002) labeled vesicles are induced by starvation in HeLa cells. HeLa cells were incubated in a regular DMEM medium (Left: Control) or in 1X HBSS buffer with 5% serum (Right: Autophagy Treatment) for 16 hours. Both control cells and starved cells were incubated with PhagyGreen™ 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 (Cat#17530, blue).

Table 2.13 Autophagy Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (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

Cell Proliferation

3.1 Cell Cycle Assays

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

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

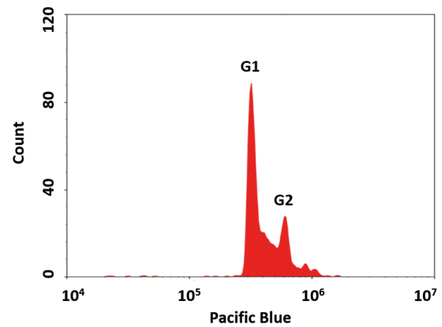

Figure 3.1. DNA profile in growing Jurkat cells. Jurkat cells were stained with Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Optimized for 405 nm Violet Laser Excitation* (Cat# 22845) for 30 minutes. The fluorescence intensity was measured using ACEA NovoCyte flow cytometer with the channel of Pacific Blue. In growing Jurkat cells, G0/G1 and G2/M phase histogram peaks are separated by the S-phase distribution.

Our Cell Meter™ Fluorimetric Cell Cycle Assay Kits (Cat# 22841, 22842 & 22845) are designed to monitor cell cycle progression and proliferation by using our proprietary cell cycle dye in permeabilized and fixed cells. The dye passes through a permeabilized membrane and intercalates into cellular DNA. The signal intensity of the cell cycle dye is directly proportional to DNA content. The percentage of cells in a given sample that are in G0/G1, S and G2/M phases, as well as the cells in the sub-G1 phase prior to apoptosis can be monitored with a flow cytometer. The kits come in three fluorescent colors, green, blue & red.

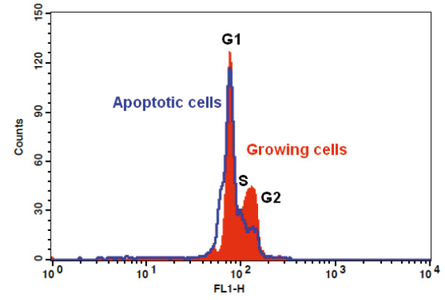

Figure 3.2. Jurkat cells were treated without (red) or with 20 μM camptothecin (blue) in a 37 °C, 5% CO_2 incubator for about 8 hours, and assayed with Cell Meter™ Fluorimetric Cell Cycle Assay kit (Cat# 22841) according to the kit instruction. The fluorescence intensity of Nuclear Green™ LCS1 (Component A) was measured with a FACSCalibur™ flow cytometer using the FL1 channel. In growing Jurkat cells, nuclei stained with Nuclear Green™ LCS1 showed G1, S, and G2 phases (red). In camptothecin treated apoptotic cells (B), the fluorescence intensity of Nuclear Green™ LCS1 was decreased, and both S and G2 phases were diminished.

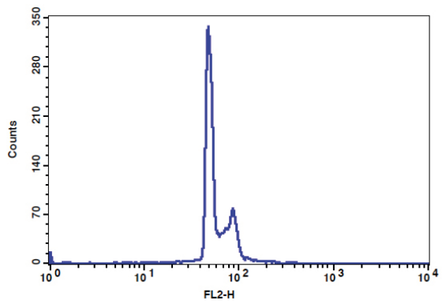

Figure 3.3. Jurkat cells were fixed and dye-loaded with Cell Meter™ Fluorimetric Cell Cycle Assay Kit (Cat# 22842) and RNase A for 30 minutes. The fluorescence intensity of Nuclear Red™ CCS1 (Component A) was measured with the FACSCalibur™ (Becton Dickinson, San Jose, CA) flow cytometer using the FL2 channel.

Table 3.1 Cell Cycle Assays

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22841	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests	503	526
22845	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Optimized for 405 nm Violet Laser Excitation*	100 tests	401	459
22842	Cell Meter™ Fluorimetric Cell Cycle Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 tests	535	617

3.2 CytoTell™ Dyes

CytoTell™ Green & CytoTell™ UltraGreen

Flow cytometry combined with fluorescence staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes, CFSE is the preferred cell proliferation indicator that is widely used for live cell analysis. However, there are a few severe problems associated with the use of CFSE for monitoring cell proliferation. 1) CFSE is highly toxic to cells since CFSE indiscriminately reacts with all amino groups, thus affects many critical intracellular protein functions (such as cell membrane GPCRs); 2) CFSE has slow response and is inconvenient to use. The CFSE fluorescence intensity of the 2nd generation cells is decreased more than 10 fold from the 1st generation. You would have to wait for another generation to start the cell proliferation analysis; 3) Medium removal is required. You would have to remove medium for cell analysis with a flow cytometer since CFSE reacts with medium components.

CytoTell™ Green (Cat# 22253) is developed to eliminate the above CFSE limitations. CytoTell™ Green can also be used for long term tracking of labeled cells. Analysis using two-parameter plots may provide better resolution of each generation, especially between undivided cells and the first generation. Cells labeled with CytoTell™ Green may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers. CytoTell™ Green can be excited by the 488 nm blue laser line with the peak emission at 520 nm, which makes it compatible with the FITC filter set.

Key Features of CytoTell™ Green

- *Spectrally similar to CFSE and FITC*
- *Much faster response to cell proliferation than CFSE*
- *More convenient to use than CFSE*
- *More sensitive than CFSE*
- *Much more stable than CFSE*

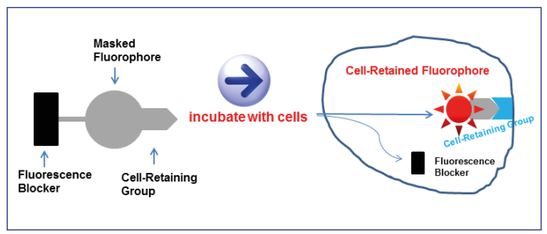

Figure 3.4. CytoTell™ dye working principle. CytoTell™ dye consists of three components: a). fluorescence blocker; b). masked fluorophore; and c). cell-retaining moiety. Upon entering live cells, the fluorescence of CytoTell™ dye is released via the removal of fluorescence blocker, and the released fluorophore is retained in cells through the cell-retaining group.

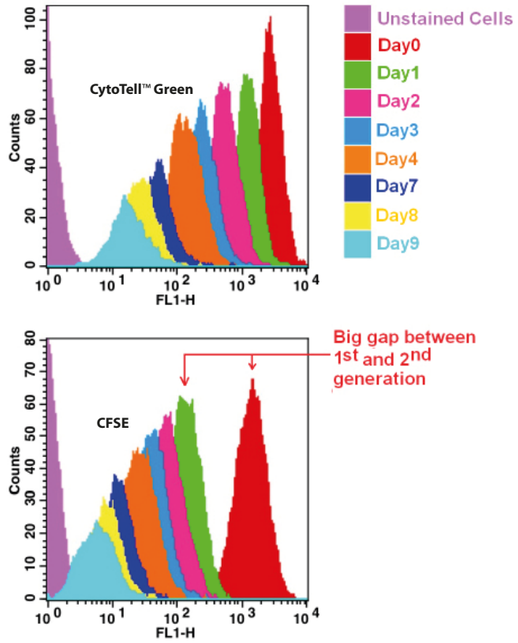

Figure 3.5. Cell tracking assays using CytoTell™ Green (Cat# 22253) and CFSE (Cat# 22022). Jurkat cells (~2x10^6 cells/mL) were stained with CytoTell™ Green or CFSE (0.5 μM) on Day 0. The cells were passed serially at 1:1 ratio for 9 days. Fluorescence intensity was measured with FACSCalibur™ flow cytometer (BD, San Jose, CA) using FL1 channel on the day after passage. Successive generations were represented by different colors.

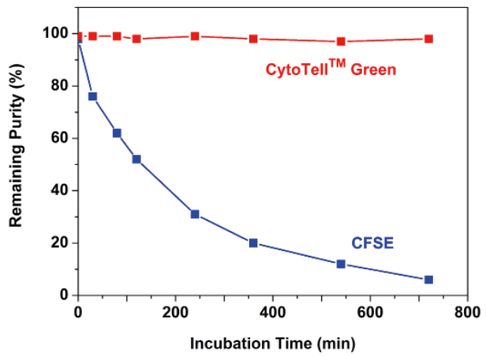

Figure 3.6. Stability comparison of CytoTell™ Green (Cat# 22253) and CFSE (Cat# 22022). 5 mM PBS working solutions of CytoTell™ Green and CFSE were monitored using HPLC (pH 7.2).

Based on our customers' feedbacks on our CytoTell™ Green, CytoTell™ UltraGreen (Cat# 22240) is our newest improvement. It has distinct advantages. 1) CytoTell™ UltraGreen is well retained in cells; 2) CytoTell™ UltraGreen exhibits much faster response and is more convenient to use than CFSE. The fluorescence intensity gap between 1st and 2nd generation is significantly minimized. As cells divide, CytoTell™ UltraGreen is distributed equally between daughter cells that can be measured as successive halving of the fluorescence intensity of the dye; 3) CytoTell™ UltraGreen is more sensitive than CFSE. Up to 9 generations may be visualized; 4) CytoTell™ UltraGreen is much more stable than CFSE. CytoTell™ UltraGreen stock solution can be stored at room temperature for a few days.

CytoTell™ UltraGreen can also be used for long term tracking of labeled cells. Analysis using two-parameter plots may provide better resolution of each generation, especially between undivided cells and the first generation. Cells labeled with CytoTell™ UltraGreen may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers. CytoTell™ UltraGreen has a peak excitation of 519 nm and can be excited by the blue (488 nm) laser line, making it compatible with FITC filter set.

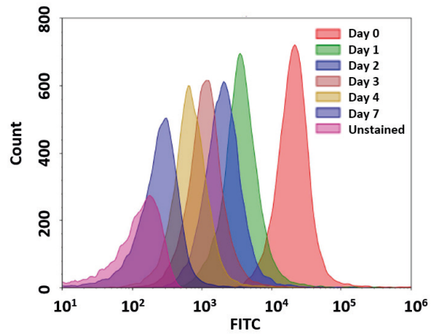

Figure 3.7. Cell tracking assay using CytoTell™ UltraGreen (Cat#22240). Jurkat cells (~2 x 106 cells/mL) were stained with CytoTell™ UltraGreen on Day 0. Cells were passed serially at 1:1 ratio for 7 days. Fluorescence intensity was measured using ACEA NovoCyte flow cytometer in FITC channel. Successive generations were represented by different colors.

CytoTell™ Blue

Flow cytometry combined with fluorescence staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes, CFSE is the preferred cell proliferation indicator that is widely used for live cell analysis. However, it is impossible to use CFSE and its fluorescein analogs for GFP-transfected cells or for the applications where a FITC-labeled antibody is used since CFSE and its fluorescein analogs have the excitation and emission spectra almost identical to those of GFP or FITC. CytoTell™ dyes are well excited with major laser lines such as 405 nm, 488 nm or 633 nm laser line with multicolor emissions. They have minimal cytotoxicity and are used for the multicolor applications with either GFP cell lines or FITC-labeled antibodies since they have either excitation or emission spectra distinct from those of fluorescein. CytoTell™ Blue is a blue fluorescent dye that stains cells evenly. It has a peak excitation of 405 nm and can be excited by the 405 nm violet laser line. Its peak emission of 450 nm can be detected with a 450/20 nm band pass filter (equivalent to Pacific Blue® or BD Horizon® V450), making it compatible with applications that use GFP or FITC antibodies for multicolor cell analysis.

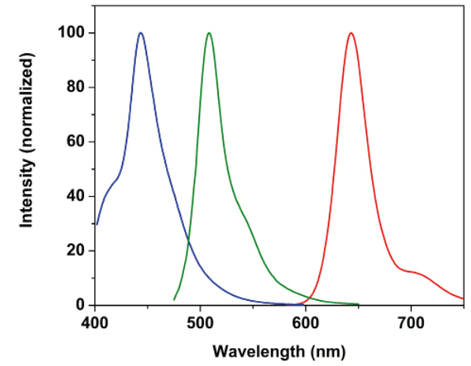

Figure 3.8. Emission spectral comparison of CytoTell™ Blue (Ex/Em = 403/454 nm, Cat# 22251), CytoTell™ Green (Ex/Em = 511/525 nm, Cat# 22253), and CytoTell™ Red (Ex/Em = 628/643 nm, Cat# 22255) in PBS buffer (pH 7.2).

CytoTell™ Red 590 & CytoTell™ Red 650

CytoTell™ Red 650 (Cat# 22255) & CytoTell™ Red 590 (Cat# 22261) are red fluorescent dyes that stain cells evenly. As cells divide, the dye is distributed equally between daughter cells that can be measured as successive halving of the fluorescence intensity of the dye. Up to 8 generations of cells may be visualized using CytoTell™ Red 650 & CytoTell™ Red 590. CytoTell™ Red 650 & CytoTell™ Red 590 can also be used for the long term tracking of labeled cells.

CytoTell™ Red 650 has a peak excitation of 630 nm and can be well excited by the 633 nm red laser line. It has a peak emission of 660 nm and can be detected with a 660/20 nm band pass filter (equivalent to APC, Alexa Fluor® 647 or Cy5®), making it compatible with the applications that use GFP or FITC antibodies for multicolor cell analysis.

CytoTell™ Red 590 has a peak excitation of 570 nm and can be excited by the yellow (561 nm) laser line. It has a peak emission of 590 nm and can be detected with a 610/20 band pass filter, making it compatible with applications that utilize GFP or FITC antibodies for multicolor cell analysis.

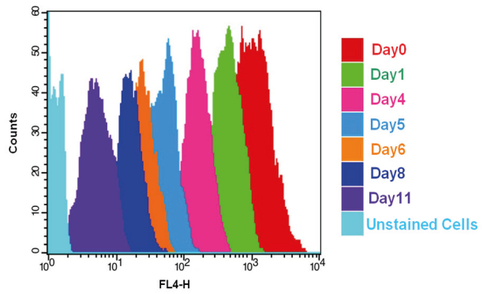

Figure 3.9. Cell tracking assay using CytoTell™ Red 650 (Cat# 22255). Jurkat cells (~ 2x10^6 cells/mL) were stained with CytoTell™ Red 650 (2 µM) on Day 0. The cells were passed serially at 1:1 ratio for 11 days. Fluorescence intensity was measured with FACSCalibur™ flow cytometer (BD, San Jose, CA) in FL4 channel on the day after passage. Successive generations were represented by different colors.

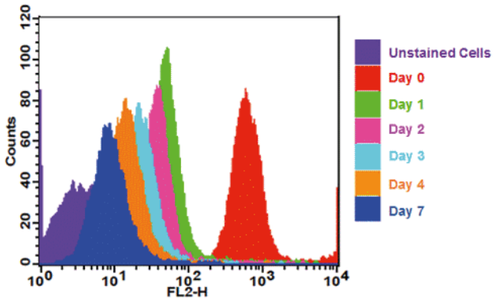

Figure 3.10. Cell tracking assay using CytoTell™ Red 590 (Cat# 22261). Jurkat cells (~ 2x10^6 cells/mL) were stained with CytoTell™ Red 590 on Day 0. The cells were passed serially at 1:1 ratio for 7 days. Fluorescence intensity was measured with FACS Calibur flow cytometer in FL2 channel. Successive generations were represented by different colors.

Table 3.2 Cell Proliferation Probes

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22022	CFSE [5-(and 6)-carboxyfluorescein diacetate, succinimidyl ester] *Mixed Isomers*	25 mg	494	521
22251	CytoTell™ Blue	500 tests	403	454
22253	CytoTell™ Green	500 tests	511	525
22257	CytoTell™ Orange	500 tests	542	556
22261	CytoTell™ Red 590	500 tests	560	574
22255	CytoTell™ Red 650	500 tests	628	643
22240	CytoTell™ UltraGreen	500 tests	592	519
22028	ReadiUse™ CFSE [5-(and 6)-Carboxyfluorescein diacetate, succinimidyl ester]	5 x 500 µg	494	521

Table 3.3 Other Related Probes

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22003	Calcein, AM *UltraPure grade* *CAS# 148504-34-1*	1 mg	495	515
22007	Calcein Blue, AM *CAS# 168482-84-6*	1 mg	360	445
21902	Calcein Deep Red™	1 mg	646	659
22008	Calcein Orange™, Sodium Salt	1 mg	525	550
21900	Calcein Red™, AM	1 mg	560	574
21908	Calcein UltraBlue™, AM	10 x 50 µg	360	445
22012	CytoCalcein™ Violet 450 *Excited at 405 nm*	1 mg	408	450
22013	CytoCalcein™ Violet 500 *Excited at 405 nm*	1 mg	410	500
22017	CytoTrace™ Green CMFDA	1 mg	494	521
22014	CytoTrace™ Orange CMTMR *CAS# 323192-14-9*	10 x 50 µg	541	565
22016	CytoTrace™ Red CFDA	1 mg	560	574
22015	CytoTrace™ Red CMTPX	10 x 50 µg	577	602

3.3 BrdU DNA Synthesis Assay

During the S phase of the cell cycle (DNA synthesis) BrdU is incorporated into the newly synthesized DNA and can be readily detected by anti-BrdU specific antibodies. In addition to DNA increases, levels of certain proteins also rise as a result of cell proliferation. For example, Ki67 is an antigen that is expressed in the nucleus of dividing cells. However, during the G0 phase of the cell cycle it is not detected. Ki67 can be combined with other proliferation markers such as BrdU and CytoTell cell stains for added confidence. These markers can also be combined with cell surface and other types of markers to gain additional information about cell subsets and their signaling pathways.

This close association between DNA synthesis and cell doubling makes the measurement of DNA synthesis very attractive for assessing cell proliferation. If labeled DNA precursors are added to the cell culture, cells that are about to divide incorporate the labeled nucleotide into their DNA. Traditionally, those assays involve the use of radiolabeled nucleosides, particularly tritiated thymidine. The amount of tritiated thymidine incorporated into the cellular DNA is quantitated by liquid scintillation counting.

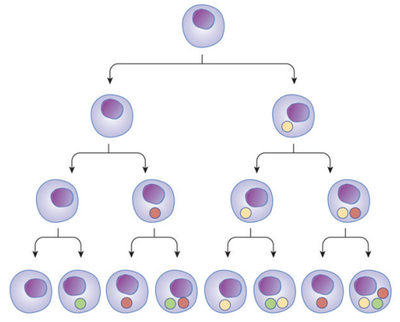

Figure 3.11. Cell proliferation exhibits a close association between DNA synthesis and cell doubling.

Experiments have shown that the thymidine analogue 5-bromo-2'-deoxy-uridine is incorporated into cellular DNA like thymidine. The incorporated BrdU could be detected by a quantitative cellular enzyme immunoassay using monoclonal antibodies directed against BrdU. The use of BrdU for such proliferation assays circumvents the disadvantages associated with the radioactive compound tritiated thymidine.

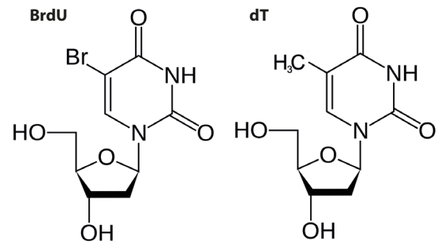

Figure 3.12. The structural similarity of thymidine (left) and 5-bromo-2´-deoxyuridine (right).

Our Cell Meter™ BrdU Cell Proliferation Assay Kit (Cat# 22270) required no harvesting of the cells; the complete assay from the start of the microculture to data analysis by an ELISA plate reader was performed in the same microplate.

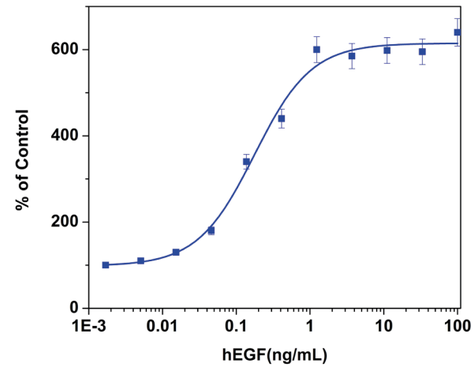

Figure 3.13. Effect of human epidermal growth factor (hEGF) on cell proliferation was monitored using Cell Meter™ BrdU Cell Proliferation Assay Kit (Cat# 22270). MCF 10A cells were seeded overnight at 1x10^4 cells/well in a 96-well Costar black wall/clear bottom plate. Cells were starved in serum free medium overnight. Different doses of hEGF were added to the cell plate and incubated for another 24 hours. Finanlly, 10 µM BrdU was added to the plate and incubated for 4 hours.

Table 3.4 BrdU Cell Proliferation Probes and Assay Kits

Cat. #	Product Name	Size
17030	BrdU [5-Bromo-2′-deoxyuridine]	25 mg
17031	BrdUTP [5-Bromo-2′-deoxyuridine 5′-triphosphate] *10 mM in TE buffer*	100 µL
17032	BrUTP [5-Bromouridine 5′-triphosphate] *10 mM in TE buffer*	100 µL
22270	Cell Meter™ BrdU Cell Proliferation Assay Kit *Red Fluorescence*	100 tests

Cell Viability & Cytotoxicity

4.1 Cell Membrane Integrity Assays

Cell membrane integrity is the most reliable and convenient method to monitor cell cytotoxicity. Based on monitoring cell membrane integrity, Cell Meter™ Cell Viability Assay Kits provide an effective tool set for fluorescence microplate and microscopic investigations of cellular functions. The kits are suitable for proliferating and non-proliferating cells. They use our proprietary cell viability dyes. The dyes are hydrophobic compounds that easily permeate intact live cells. The fluorescence of the dyes is strongly enhanced upon entering into live cells.

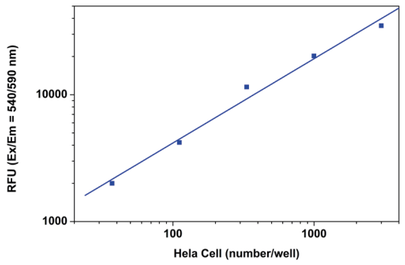

Figure 4.1. HeLa cell number response was measured with Cell Meter™ Cell Viability Assay Kit (Cat# 22783). HeLa cells at 0 to 3,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 100 µL/well of CytoCalcein™ Red dye-loading solution for 30 min at 37 °C. The fluorescence intensity was measured at Ex/Em = 540/ 590 nm (cutoff at 570 nm) with bottom read mode using Flexstation (from Molecular devices).

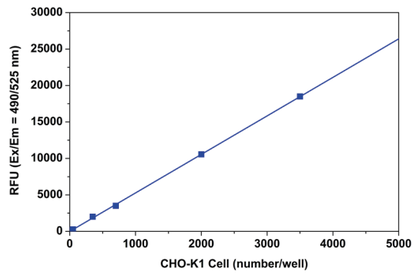

Figure 4.2. CHO-K1 cell number responses were measured with Cell Meter™ Cell Viability Assay Kit (Cat# 22786). CHO-K1 cells were seeded at 0 to 5,000 cells/well/100 µL overnight in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 100 µL/well of CytoCalcein™ Green dye-loading solution for 1 hour at 37 °C. The fluorescence intensity was measured at Ex/Em = 490/ 525 nm.

Live or Dead™ Cell Viability Assay Kit (Cat# 22789) is a dual color kit. It uses two non-fluorescent indicators, calcein AM for viable cells and a cell-impermeable DNA-binding dye for the cells with compromised membranes. Calcein AM is a hydrophobic compound that easily permeates intact live cells, and becomes strongly fluorescent upon hydrolysis by esterases. The hydrolysis of the non-fluorescent calcein AM by intracellular esterases generates the strongly fluorescent hydrophilic calcein that is well-retained in the cell cytoplasm. The esterase activity is proportional to the number of vial cells. The DNA-binding dye is quite polar and impermeable for viable cells that have intact membranes. It becomes fluorescent only upon binding to DNA of dead cells. Cells grown in black-walled plates can be stained and quantified in less than two hours. The assay is more robust and accurate than the other viability assays. It can be readily adapted for high-throughput assays in a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry.

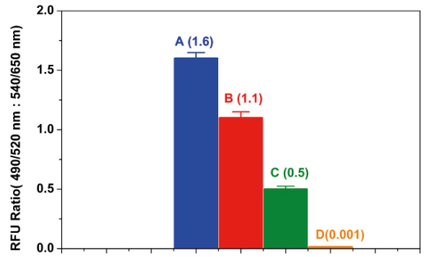

Figure 4.3. The effect of saponin on cell viability in Jurkat cells measured with Cell Meter™ Cell Viability Assay Kit (Cat# 22789). Jurkat cells were treated with or without 0.5% saponin at 2X10^6 cells/mL for 5 minutes. The cells were centrifuged and the supernatant were replaced with fresh medium. 100 µL of untreated cells (A), 50 uL of untreated and 50 µL of treated cells (B), 25 uL of untreated and 75 uL treated cells (C), and 100 µL of 0.5% saponin treated cells (D) were plated in a 96-well black wall/clear bottom Poly-D-lysine plate. The cells were incubated with 100 µL/well of CytoCalcein™ Green/ propidium iodide dye-loading solution for 1 hour at 37 °C. The fluorescence intensity ratio of 520 nm (excited at 490 nm) to 650 nm (excited at 540 nm) on live and dead cells was showed as indicated (n=6).

All the Cell Meter™ Cell Viability Assay Kits come with sufficient reagents to perform either 200 assays (96-well format) or 800 assays (384-well format). They provide all the essential components with an optimized protocol for the cell viability analysis. These Cell Meter™ Cell Viability Assay Kits can be used for both proliferating and non-proliferating cells. They are useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity.

Table 4.1 Cell Viability Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22785	Cell Meter™ Cell Viability Assay Kit *Blue Fluorescence*	500 tests	360	445
22784	Cell Meter™ Cell Viability Assay Kit *Blue Fluorescence with 405 nm Excitation*	500 tests	405	450
22786	Cell Meter™ Cell Viability Assay Kit *Green Fluorescence*	500 tests	495	515
22787	Cell Meter™ Cell Viability Assay Kit *NIR Fluorescence Optimized for Fluorescence Microplate Reader*	200 tests	646	659
22783	Cell Meter™ Cell Viability Assay Kit *Red Fluorescence*	200 tests	560	574
22789	Live or Dead™ Cell Viability Assay Kit *Green/Red Dual Fluorescence*	200 tests	492/540	515/620

4.2 Cellular Nucleic Acid Detection

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

Labeling the Nuclei of Live Cells

Both Hoechst 33258 (Cat# 17520) and Hoechst 33342 (Cat# 17530) are quite soluble in water and relatively nontoxic. They are cell membrane–permeant, minor groove–binding DNA stains that fluoresce bright blue upon binding to DNA. Hoechst 33342 has slightly higher membrane permeability than Hoechst 33258. These Hoechst dyes, which can be excited with the UV spectral lines of the argon-ion laser and by most conventional fluorescence excitation sources, exhibit relatively large Stokes shifts (excitation/emission maxima ~350/460 nm), making them suitable for multicolor labeling experiments. Hoechst 34580 can be better excited by violet laser at 405 nm.

DAPI (Cat# 17510) is quite soluble in water but has limited solubility in PBS buffer. We offer both DAPI chloride and lactate salt. DAPI is an excellent nuclear counterstain, showing a distinct banding pattern in chromosomes. It is one of the most common nuclear dyes for staining nuclei in lives cells in combination with fluorescence imaging or flow cytometry. DAPI demonstrates blue fluorescence upon binding to DNA and can be excited with a mercury-arc lamp or with the UV lines of the argon-ion laser. Binding of DAPI to dsDNA produces ~20-fold fluorescence enhancement, apparently due to the displacement of water molecules from both DAPI and the minor groove.

LDS 751 (Cat# 17561) has its peak excitation at ~543 nm on dsDNA. It can be excited by the argon-ion laser at 488 nm and is particularly useful in multicolor analyses due to its long-wavelength emission maximum (~712 nm). Binding of LDS 751 to dsDNA results in ~20-fold fluorescence enhancement. LDS 751 is a cell-permeant nucleic acid stain that has been used to discriminate intact nucleated cells from nonnucleated and damaged nucleated cells, as well as to identify distinct cell types in mixed populations of neutrophils, leukocytes and monocytes by flow cytometry.

Nuclear Green™ LCS1 (Cat# 17540), Nuclear Orange™ LCS1 (Cat# 17541), Nuclear Red™ LCS1 (Cat# 17542) and Nuclear Yellow (Cat# 17539) are fluorogenic, DNA-selective and cell-permeant dyes for analyzing DNA content in living cells. The fluorescence of these dyes is significantly enhanced upon binding to DNA. They can be used in fluorescence imaging, microplate and flow cytometry applications. These DNA-binding dyes might be used for multicolor analysis of live cells with proper filter sets.

Our recently developed Nuclear Blue™ LCS1 (Cat# 17543) is a fluorogenic, DNA-selective and cell-permeant dye for analyzing DNA content in living cells. The Nuclear Blue™ LCS1 has its blue fluorescence significantly enhanced upon binding to DNA. It can be used in fluorescence imaging, microplate and flow cytometry applications. It is well excited by violet laser at 405 nm, and emits blue/cyan fluorescence light with an emission maximum at ~440 nm, and provides an excellent tool for flow cytometers equiped with a 405 nm violet laser source. This DNA-binding dye might be used for multicolor analysis of live cells with the filter sets of Pacific Blue™ and BD Horizon™ V450.

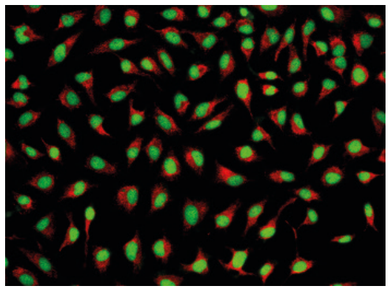

Figure 4.4. Image of live HeLa cells stained with Nuclear Green™ LCS1 (Cat# 17540). The mitochondria of live HeLa cells were stained with red fluorescence Cell Navigator™ Mitochondrion Staining Kit (Cat# 22668).

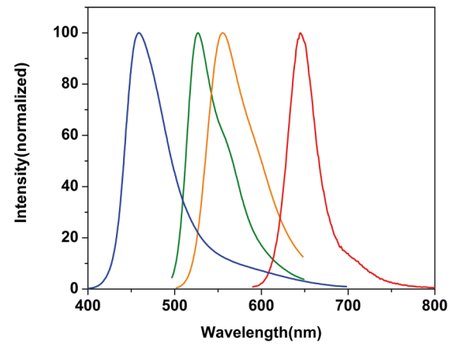

Figure 4.5. The normalized emission spectral comparison of Nuclear Blue™ LCS1 (Ex/Em = 401/459 nm, Cat# 17543), Nuclear Green™ LCS1 (Ex/Em = 503/526 nm, Cat# 17540), Nuclear Orange™ LCS1 (Ex/Em = 514/555 nm, Cat#17541), and Nuclear Red™ LCS1 (Ex/Em = 622/645 nm, Cat# 17542) bound to calf thymus DNA.

Labeling the Nuclei of Dead Cells

Propidium iodide (PI) is cell-impermeable and commonly used for identifying dead cells in a population of cells and as a counterstain in multicolor fluorescent techniques. It can also be used to differentiate necrotic, apoptotic and normal cells. The fluorescence of PI is enhanced by 20-30-fold upon binding to nucleic acids. The fluorescence excitation maximum is red-shifted by 30–40 nm and the fluorescence emission maximum blue-shifted by 15 nm or so. PI is cell-impermeable and commonly used for identifying dead cells in a population of cells and as a counterstain in multicolor fluorescent techniques.

7-Amino actinomycin D (7-AAD, Cat# 17501) is another non-permeant dye that can be used to identify non-viable cells. 7-AAD is typically used with a flow cytometer. Cells with damaged plasma membranes or with impaired/no cell metabolism are unable to prevent the dye from entering the cell. Once inside the cell, the dyes bind to intracellular DNA producing highly fluorescent adducts which identify the cells as non-viable. 7-AAD is well excited by the 488 nm laser line of an argon laser with fluorescence detected above 650 nm. Although the emission intensity of 7-AAD is lower than that of PI, the longer wavelength emission may make it more useful for multiplexing assays in combination with other 488 nm-excited fluorochromes such as FITC and PE.

Nuclear Green™ DCS1 (Cat# 17550), Nuclear Orange™ DCS1 (Cat# 17551) and Nuclear Red™ DCS1 (Cat# 17552) are fluorogenic, DNA-selective and cell-impermeant dyes for analyzing DNA content in dead, fixed or apoptotic cells. As the LCS1 reagents, the fluorescence of the DCS1 dyes is significantly enhanced upon binding to DNA. They can be used in fluorescence imaging, microplate and flow cytometry applications. These DNA-binding dyes might be used for multicolor analysis of dead, fixed or apoptotic cells with proper filter sets.

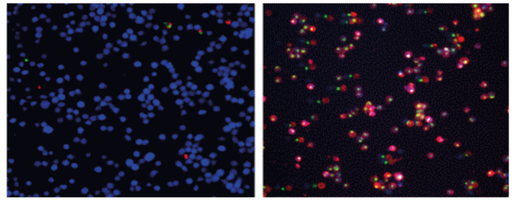

Figure 4.6. Binding activity of Apopxin™ Deep Red to phosphatidylserine in Jurkat cells. The fluorescence images demonstrated that live cells (blue) were stained by CytoCalcein™ Violet 450 (Cat# 22012), apoptotic cells (red) were stained by Apopxin™ Deep Red, and necrotic cells (green) were stained by Nuclear Green™ DCS1 (Cat# 17550). Apoptosis was induced by 1µM staurosporine for 3 hours. The fluorescence images of the cells were taken with Olympus fluorescence microscope using the violet, Cy5® and FITC channel respectively. A: Non-induced control cells; B: Triple staining of staurosporine-induced cells.

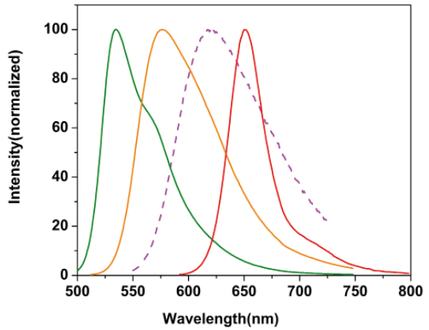

Figure 4.7. The normalized emission spectral comparison of Nuclear Green™ DCS1 (Ex/Em = 503/526 nm, Cat# 17550), Nuclear Orange™ DCS1 (Ex/Em = 514/555 nm, Cat# 17551), and Nuclear Red™ DCS1 (Ex/Em = 622/645 nm, Cat# 17552) in the presence of calf thymus DNA. The dotted line is emission spectrum of propidium iodide bound to DNA (Ex/Em = 535/617 nm, Cat# 17515).

Table 4.2 Cell Nuclear Stains

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

4.3 Cell Cytotoxicity Assays

Monitoring cell cytotoxicity is one of the most essential tasks for studying cellular functions. Cell Meter™ Cytotoxicity Assay Kits (Cat# 22780 & 22781) are a set of tools for monitoring cell cytotoxicity. Cell Meter™ Colorimetric Cell Cytotoxicity Kit (Cat# 22780) uses proprietary water-soluble dyes that change the absorption spectra upon cellular reduction. The absorption ratio change is directly proportional to the number of living cells. This kit does not require pre-mixing of components and has higher sensitivity compared to the tetrazolium based colorimetric assays (such as MTT and XTT).

Cell Meter™ Cell Cytotoxicity Assay Kits are more sensitive for cell proliferation and cytotoxicity than other assays such as MTT. The kit components are quite stable with minimal cytotoxicity, thus a longer incubation time (such as 24 to 48 hours) is possible if required. The characteristics of the high sensitivity (<100 CHO cells), non-radioactivity and no-wash method make the kits suitable for high throughput screening of cell proliferation or cytotoxicity against a variety of compounds. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format.

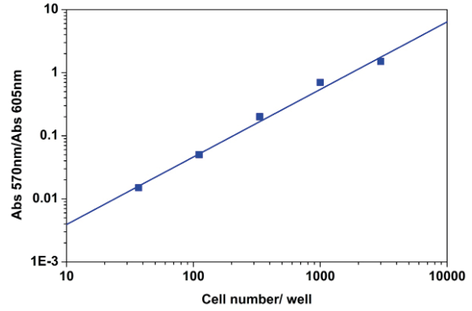

Figure 4.8. CHO-K1 cell number responses were measured with Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit (Cat# 22780). CHO-K1 cells were seeded at 0 to 10,000 cells/well/100 µL overnight in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 20 µL/well of Component A for 3 hours at 37 °C. The absorbance intensity was measured at 570 nm and 605 nm with SpectraMax plus (Molecular Devices). The ratio of OD_{570}/OD_{605} is proportional to the number of cells as indicated.

Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit (Cat# 22781) provides a fast, simple, accurate and homogeneous assay for the fluorimetric detection of viable cells. This assay is based on the observation that oxidized non-fluorescent blue resazurin is reduced to a red fluorescent dye (resorufin) by accepting an electron from mitochondrial respiratory chain in live cells. The amount of resorufin produced is directly proportional to the number of living cells.

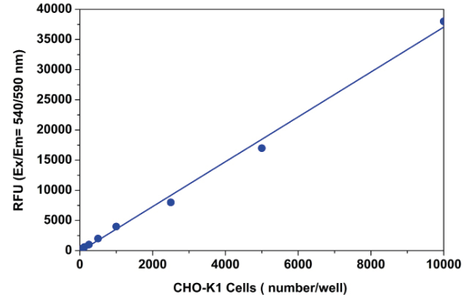

Figure 4.9. CHO-K1 cell number responses were measured with Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit (Cat# 22781). CHO-K1 cells were seeded at 0 to 10,000 cells/well/100 µL overnight in a 96-well black wall/clear bottom Costar plate. The cells were incubated with 20 µL/well of Component A for 3 hours at 37 °C. The fluorescence intensity was measured at Ex/Em = 540/590 nm with NOVOstar instrument (BMG Labtech). The fluorescence intensity was linear (R^2 = 0.998) to the cell number as indicated. The detection limit is 60 cells/well (n=6).

Cytotoxicity Assays: Cytotoxicity assays are widely used to screen cytotoxicity in compound libraries. Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Vital dyes, such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells. However, if the cell membrane is compromised, they freely cross the membrane and stain intracellular components. Alternatively, membrane integrity can be assessed by monitoring the passage of substances that are normally sequestered inside cells to the outside. One molecule, lactate dehydrogenase (LDH), is commonly measured using LDH assay. Cytotoxicity can also be monitored using a redox indicator. Viable cells reduce the MTS reagent to a colored formazan product. A similar redox-based assay has also been developed using the fluorescent dye, resazurin. In addition to using dyes to indicate the redox potential of cells in order to monitor their viability, researchers have developed assays that use ATP content as a marker of viability. Such ATP-based assays include bioluminescent assays in which ATP is the limiting reagent for the luciferase reaction.

Table 4.3 Cell Cytotoxicity Assay Kits

Cat. #	Product Name	Size	Ex (nm)	Em (nm)
22780	Cell Meter™ Colorimetric Cell Cytotoxicity Assay Kit	1,000 tests	575	N/A
22781	Cell Meter™ Fluorimetric Cell Cytotoxicity Assay Kit	1,000 tests	571	585
21610	PhosphoWorks™ Luminometric ATP Assay Kit *Bright Glow*	1 plate	N/A	560
21612	PhosphoWorks™ Luminometric ATP Assay Kit *DTT-Free*	1 plate	N/A	560

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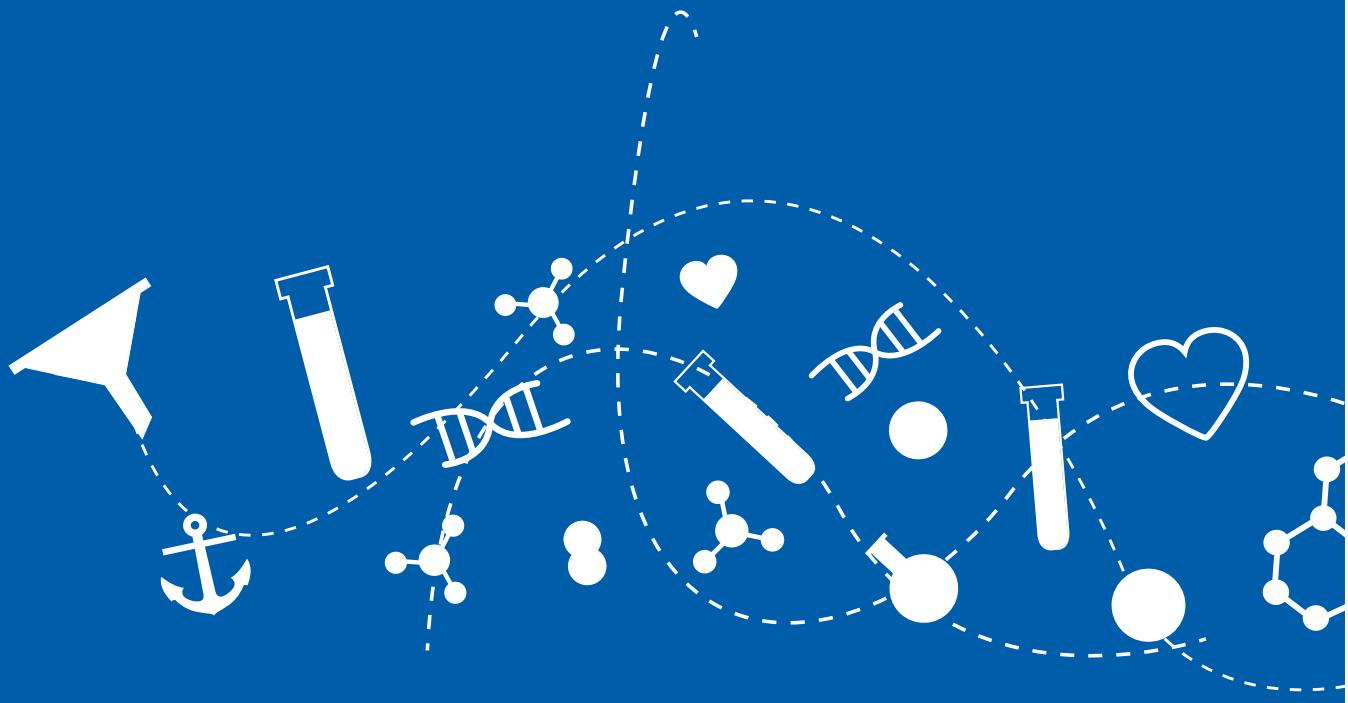

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