Assays for

Cell Viability & Cell Death

Biotium

Glowing Products for Science™

Mitochondrial Membrane Potential and Cellular Glutathione

MitoView™ Dyes

Loss of mitochondrial membrane potential is a hallmark for apoptosis. Biotium offers our MitoView™ Blue and MitoView™ 633 dyes for membrane potential-sensitive staining of mitochondria. We also offer MitoView™ Green, a membrane-potential independent mitochondrial dye that can be used to image mitochondria following mitochondrial depolarization, or after fixation.

JC-1 and other mitochondrial dyes

In healthy cells, JC-1 dye aggregates in mitochondria as a function of membrane potential, resulting in red fluorescence with brightness proportional to the membrane potential. Conversely, in apoptotic and necrotic cells with diminished mitochondrial membrane potential, JC-1 exists in a green fluorescent monomeric form in the cytosol, allowing of cell viability to be assessed by measuring the ratio of red to green fluorescence by flow cytometry or fluorescence plate reader.

We also offer a selection of classic potentiometric mitochondrial stains in a variety of fluorescent colors.

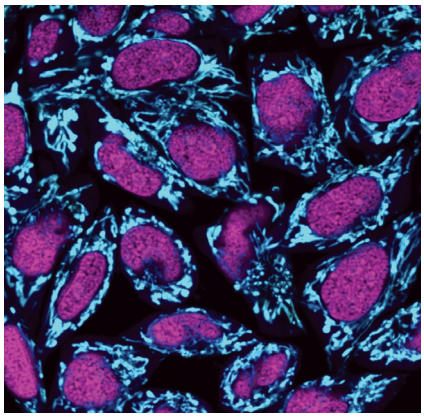

Figure 1. HeLa cells stained with MitoView™ Blue (cyan) and RedDot™1 far-red nuclear stain (magenta).

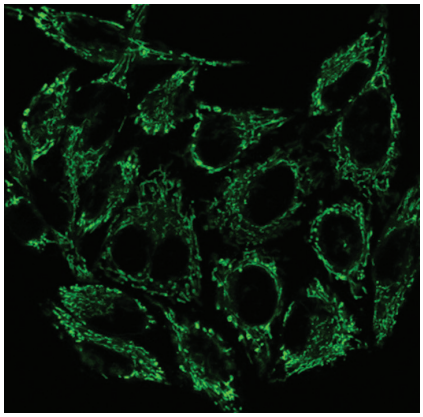

Figure 2. HeLa cells stained with MitoView™ Green.

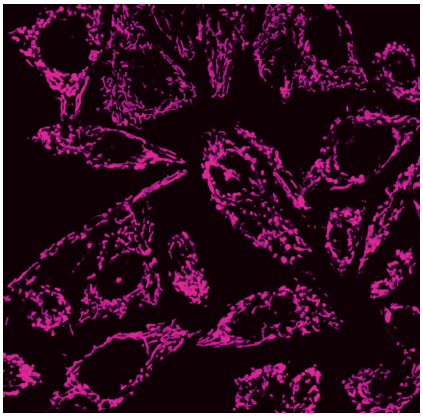

Figure 3. HeLa cells stained with MitoView™ 633.

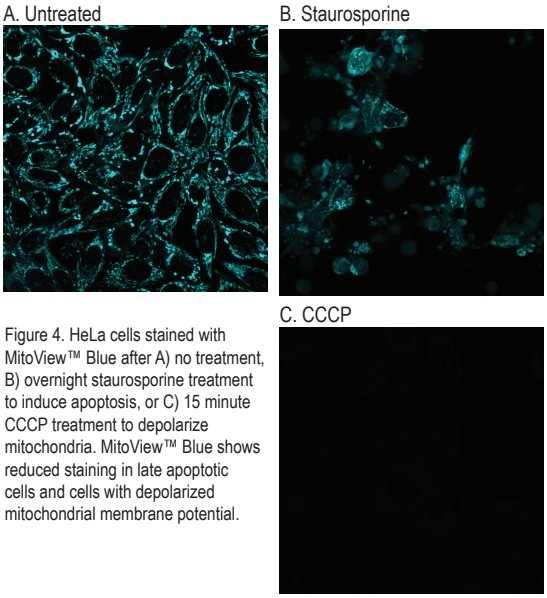

Figure 4. HeLa cells stained with MitoView™ Blue after A) no treatment, B) overnight staurosporine treatment to induce apoptosis, or C) 15 minute CCCP treatment to depolarize mitochondria. MitoView™ Blue shows reduced staining in late apoptotic cells and cells with depolarized mitochondrial membrane potential.

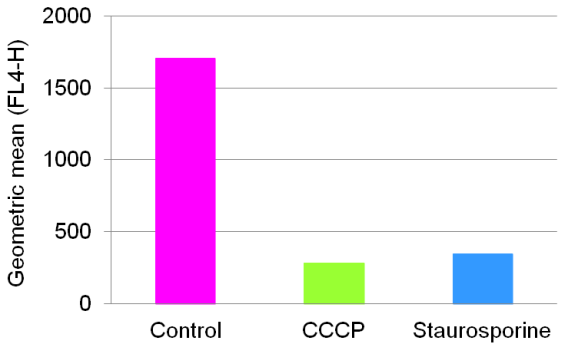

Figure 5. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in decreased MitoView™ 633 staining.

MCB Glutathione Detection Kit

Diminished cellular glutathione (GSH) level occurs during apoptosis due to GSH efflux from mitochondria. Monochlorobimane (MCB), which reacts with thiols to form a blue fluorescent product, allowing fluorometric quantitation of GSH in cell lysates.

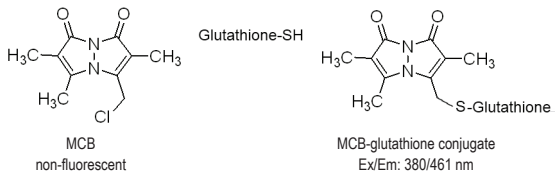

Figure 6. MCB glutathione assay principle.

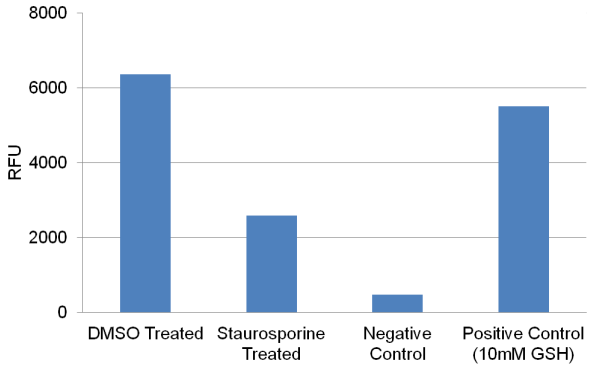

Figure 7. Jurkat cells were treated with DMSO (Control) or induced to undergo apoptosis by treatment with 1 uM staurosporine for 5 hours. Glutathione levels were measured using the MCB Glutathione Detection Kit by fluorescence microplate reader.

MitoView™ Dyes	Color	Ex/Em	Mitochondrial Membrane Potential Dependent?	Catalog No.	Size
MitoView™ Blue	Blue	398/440 nm	Yes	70052	20 x 50 ug
MitoView™ 633	Far-red	622/648 nm	Yes	70055	20 x 50 ug
MitoView™ Green	Green	490/523 nm	No	70054	20 x 50 ug

Other Mitochondrial Dyes	Color	Ex/Em	Mitochondrial Membrane Potential Dependent?	Catalog No.	Size
JC-1, chloride salt	Green/ Red	510/527 nm (cytoplasm) 585/590 nm (polarized mitochondria)	Two-color detection mitochondria polarization/depolarization	70011	5 mg
JC-1, iodide salt	Green/ Red	510/527 nm (cytoplasm) 585/590 nm (polarized mitochondria)	Two-color detection mitochondria polarization/depolarization	70014	5 mg
Rhodamine 123	Green	505/534 nm	Yes	70010	50 mg
TMRE	Red	548/573 nm	Yes	70016	25 mg
TMRE, 2 mM in DMSO	Red	548/573 nm	Yes	70005	0.5 mL
TMRM	Red	548/573 nm	Yes	70017	25 mg
DASPEI	Red	461/589 nm	Yes	70018	100 mg
$DiIC_1(5)$	Far-red	638/658 nm	Yes	70015	100 mg

Assay Kits	Color	Ex/Em	Assay	Catalog No.	Size
NucView™ 488 and MitoView™ 633 Apoptosis Kit	Green/ Red	500/530 nm (caspase-3) 622/648 nm (polarized mitochondria)	Two color detection caspase-3 activity and mitochondrial potential; see page 5 for more details	30062	100 assays
JC-1 Mitochondrial Membrane Potential Detection Kit	Green/ Red	510/527 nm (cytoplasm) 585/590 nm (polarized mitochondria)	Two-color detection mitochondria polarization/depolarization	30001	100 assays
MCB Glutathione Detection Kit	Blue	394/490 nm	Detection of cellular glutathione	30019	100 assays

Real-time detection of caspase activity in intact cells

NucView™ Caspase-3 Substrates

Proteolysis of cellular substrates by caspase-3 results in the morphological and biochemical features of apoptosis. NucView™ Caspase-3 Substrates are novel cell membrane-permeable fluorogenic caspase substrates designed for detecting caspase-3/7 activity in real time.

Traditional fluorogenic caspase substrates require cell lysis and cannot be used to measure caspase activity in live cells. Fluorescently-labeled caspase inhibitor assay (FLICA) reagents can enter live cells to detect caspase activity, but because the fluorescent probes are also irreversible caspase inhibitors, they cannot be used to follow caspase activity in real time.

NucView™ Caspase-3 Substrates consist of a fluorogenic DNA dye and a DEVD substrate moiety recognized by caspase-3/7. The substrate, which is initially not fluorescent and nonfunctional as a DNA dye, crosses the cell membrane to enter the cytoplasm, where it is cleaved by caspase-3 to form a high-affinity DNA dye. The released dye can bind DNA, resulting in bright nuclear fluorescence (Figures 1, 2), allowing caspase-3/7 activity to be monitored in individual intact cells in real time. NucView™ substrates also can be used in a rapid and convenient homogenous end-point assay.

We offer green fluorogenic NucView™ 488 Caspase-3 Substrate and kits, validated in more than a hundred published studies and cell types. We also offer blue fluorogenic NucView™ 405 Caspase-3 Substrate for confocal microscopy or flow cytometry using the 405 nm laser line, and orange fluorogenic NucView™ 530 Caspase-3 Substrate for multi-color flexibility.

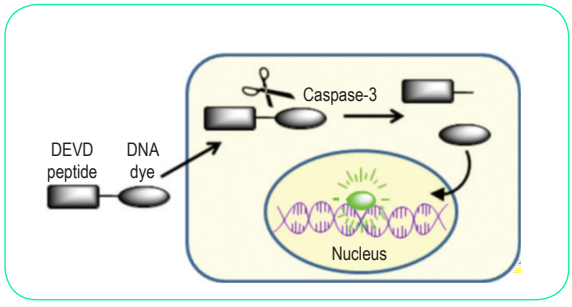

Figure 1. Principal of apoptosis detection using NucView™ Caspase-3 Substrates.

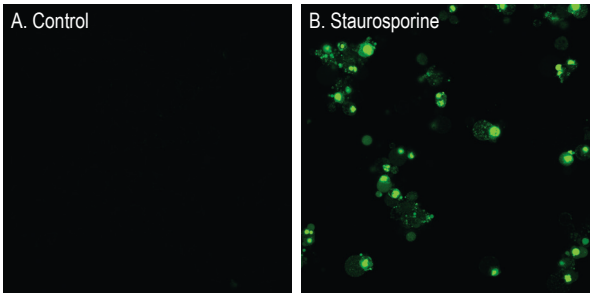

Figure 2. Imaging of apoptotic cells with NucView™ 488 Caspase-3 Substrate. Confocal fluorescence microscopy of HeLa cells incubated with NucView™ 488 Caspase-3 Substrate overnight at 37°C without additional treatment (A) or in the presence of staurosporine (B) to induce apoptosis.

NucView™ Caspase-3 Substrates

- **Bifunctional: allow caspase-3/7 detection and visualization of apoptotic nuclear morphology**
- **Do not interfere with caspase activity, allowing real time caspase monitoring**
- **Fast staining in cell culture medium with no wash required before imaging or flow cytometry**
- **Tolerant of formaldehyde fixation and permeabilization**
- **Detectable by fluorescence microscopy, flow cytometry, or fluorescence microplate reader**
- **For use in adherent or suspension cells**

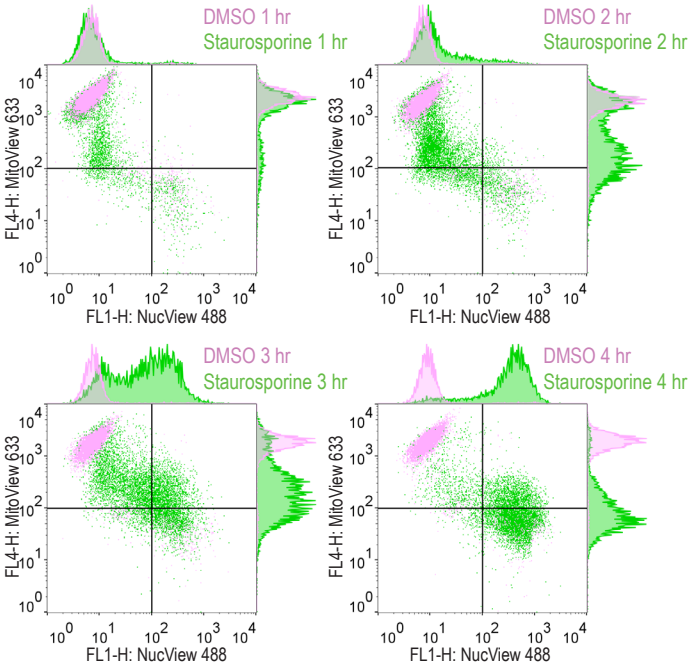

Figure 3. Flow cytometry analysis of Jurkat cells treated with staurosporine (green) to induce apoptosis, or DMSO controls (pink), using the NucView™ 488 and MitoView™ 633 Apoptosis Kit. Fluorescence was analyzed on a BD FACSCalibur flow cytometer. As apoptosis progresses over time in staurosporine-treated cells, NucView™ 488 signal (FL1, x-axis) increases and mitochondrial membrane potential measured by MitoView™ 633 staining (FL4, y-axis) decreases.

NucView™ enzyme substrate technology is covered under U.S. patents. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to btinfo@biotium.com.

Now more color options

NucView™ 488: Green fluorogenic substrate (Ex/Em 504/534 nm), tested in more than 100 cell lines and publications*

NucView™405: Blue fluorogenic substrate (Ex/Em 429/469 nm) for flow cytometry or confocal microscopy with the 405 nm laser line

NucView™ 530: Orange fluorogenic substrate (Ex/Em 528/563 nm) for microscopy or flow cytometry in the Cy®3/R-PE channel

*Based on published reports. Visit www.biotium.com to download a list of cell lines and references.

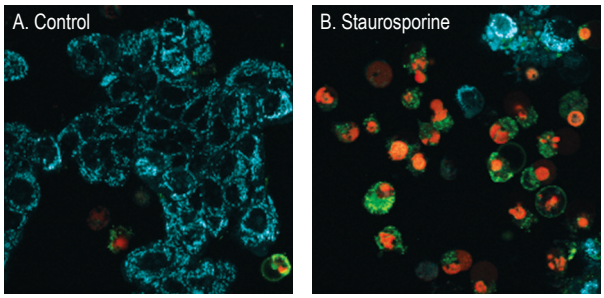

Figure 4. Detection of mitochondrial membrane potential, caspase-3 activity, and phosphatidylserine in MCF-7 cells. Cells were either untreated (A) or treated with staurosporine overnight to induce apoptosis (B), then stained with MitoView™ Blue, NucView™ 530 Caspase-3 Substrate, and CF™488A Annexin V for 30 minutes at 37°C in cell culture medium with no wash. Healthy cells show mitochondrial staining with MitoView™ Blue (cyan). Apoptotic cells lose MitoView™ Blue staining (cyan), and show NucView™ 520 staining of nuclei (red) and CF™488A Annexin V staining of cell membranes (green).

NucView™ Caspase-3 Assay Kits

NucView™488 conveniently paired with other cell death probes:

- NucView™ 488 Caspase-3 Assay Kit for Live Cells, with Ac-DEVD-CHO Caspase-3 Inhibitor
- NucView™ 488 and MitoView™ 633 mitochondrial membrane potential dye (see p. 2)
- NucView™ 488 and CF™ dye conjugated Annexin V (see p. 6)
- NucView™ 488 and RedDot™2 dead cell stain (see p. 10)

Cy Dye is a registered trademark of GE Healthcare.

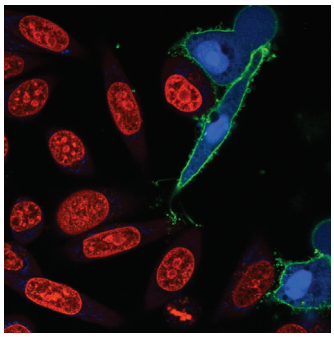

Figure 5. Imaging of apoptotic cells with NucView™ 405. HeLa cells were stained with NucView™ 405 caspase-3 substrate, CF™488A Annexin V, and RedDot™1 for 30 minutes at 37°C in cell culture medium with no wash step. NucView™ 405 signal (blue) localized to apoptotic cells, identified by CF™488A Annexin V staining (green). Nuclei were counterstained red with RedDot™ 1 far-red nuclear stain. Cells were imaged on a Zeiss LSM 700 confocal microscope.

Additional caspase substrates and inhibitors

Biotium offers rhodamine 110 (R110)-based assay kits for fluorescence or absorbance-based detection of caspase-3 activity in cell lysates, including a R110-based homogenous caspase-3 assay kits for high throughput screening by fluorescence microplate reader. Biotium also offers a coumarin (AMC)-based blue fluorogenic substrate for measuring caspase activity in cell lysates by fluorescence microplate reader.

Ac-DEVD-CHO is a competitive inhibitor of caspase-3 for use in cultured cells or cell lysates.

Caspase Substrates and Detection Kits	Catalog no.	Unit size
NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10402	100 uL
NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in PBS	10403	100 uL
NucView™ 405 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10405-T	10 uL trial size
NucView™ 405 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10405	100 uL
NucView™ 530 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10406-T	10 uL trial size
NucView™ 530 Caspase-3 Enzyme Substrate, 1 mM in DMSO	10406	100 uL
NucView™ 488 Caspase-3 Assay Kit for Live Cells	30029-T	25 assay trial size
NucView™ 488 Caspase-3 Assay Kit for Live Cells	30029	100 assays
Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™594 Annexin V	30067	50 assays
Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™640R Annexin V	30073	50 assays
NucView™ 488 and MitoView™ 633 Apoptosis Kit	30062	100 assays
NucView™ 488 and RedDot™2 Apoptosis & Necrosis Kit	30072	100 assays
Caspase-3 DEVD-R110 Fluorometric & Colorimetric Assay Kit	30008-1	25 assays
	30008-2	100 assays
Caspase-3 DEVD-R110 Fluorometric HTS Assay	30009-1	10 assays
	30009-2	100 assays
	30009-3	1000 assays
Ac-DEVD-AMC	10202	5 mg
Ac-DEVD-CHO Caspase-3 Inhibitor	10404-1	1 mg
	10404	5 mg

Annexin V Conjugates and Kits

Annexin V conjugates

Annexin V is a 35-36 kDa protein that has a high affinity for phosphatidylserine (PS). During apoptosis, PS is translocated from the inner to the outer leaflet of the plasma membrane, where it can be stained by fluorescent conjugates of Annexin V, for detection of apoptotic cells by flow cytometry (Fig. 1) or fluorescence microscopy (Fig. 2-3). Biotium offers Annexin V conjugates and kits featuring our exceptionally bright and photostable CF™ dyes. For example, our CF™488A green fluorescent Annexin V conjugate (Fig. 2) is much brighter and more photostable than the traditional FITC-Annexin V, allowing the use of 10-fold less conjugate in staining. Our near-infrared CF™ dye conjugates of Annexin V are supplied lyophilized and preservative-free, and are suitable for in vivo imaging.

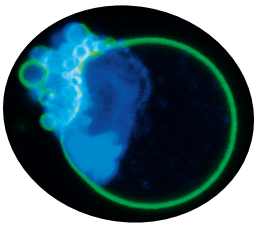

Figure 2. Apoptotic HeLa cell stained with CF™488A Annexin V (green) and NucView™ 405 (cyan). See p. 4-5 for more information in NucView™ Caspase-3 Substrates.

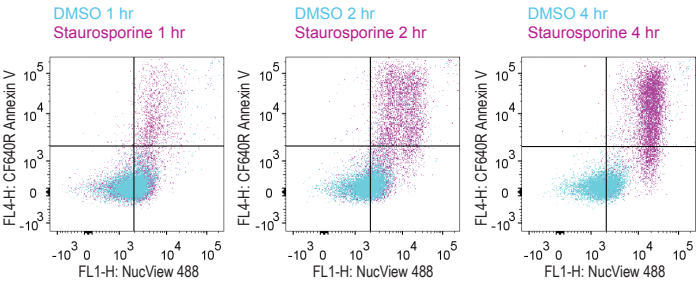

Figure 1. Jurkat cells were treated with staurosporine to induce apoptosis (pink), or with DMSO as a negative control (blue) for the times indicated, then stained for 15 minutes at room temperature with NucView™ 520 Caspase-3 Substrate (FL1-H, x-axis) and CF™640R Annexin V (FL4-H, y-axis) in cell culture medium prior to analysis using a BD LSRII flow cytometer. See pp. 4-5 for more information in NucView™ Substrates.

CF™488A-Annexin V Apoptosis Kits with PI or 7-AAD

Green fluorescent CF™488 Annexin V paired with red fluorescent propidium iodide or far-red fluorescent 7-AAD for detection of necrotic and late apoptotic cells with compromised membrane integrity by fluorescence microscopy or flow cytometry.

Apoptosis & Necrosis Quantitation Kits

CF™488A Annexin V plus Ethidium Homodimer III, a novel membrane-impermeant nucleic acid dye developed at Biotium with higher affinity for DNA and higher fluorescence quantum yield than propidium iodide. The Apoptotic, Necrotic, and Healthy Cells Quantitation Kit also includes blue fluorescent Hoechst 33342 DNA dye for visualizing the healthy cells and dead cells (Figure 3).

Dual apoptosis assay kits

Annexin V conjugated to our deep red CF™594 or far-red CF™640R dyes is offered together with NucView™ 488 Caspase-3 Substrate for simultaneous detection of caspase-3 activity and phosphatidylserine exposure by fluorescence microscopy or flow cytometry (see page 5 for more information on NucView™ substrates).

Annexin V Conjugates	Ex/Em (nm)	Catalog number	Unit size
CF™350 Annexin V, 50 ug/mL	347/448	29012	0.5 mL
CF™405M Annexin V, 50 ug/mL	408/452	29009	0.5 mL
CF™488 Annexin V, 50 ug/mL	490/515	29005	0.5 mL
CF™555 Annexin V, 50 ug/mL	555/565	29004	0.5 mL
CF™568 Annexin V, 50 ug/mL	562/583	29010	0.5 mL
CF™594 Annexin V, 50 ug/mL	593/614	29011	0.5 mL
CF™633 Annexin V, 50 ug/mL	630/650	29008	0.5 mL
CF™640R Annexin V, 50 ug/mL	642/662	29014	0.5 mL
CF™647 Annexin V, 50 ug/mL	650/665	29003	0.5 mL
CF™680 Annexin V, lyophilized	681/698	29007	25 ug
CF™750 Annexin V, lyophilized	755/777	29006	25 ug
CF™770 Annexin V, lyophilized	770/797	29046	25 ug
CF™790 Annexin V, lyophilized	784/806	29047	25 ug
FITC Annexin V, 50 ug/mL	490/525	29001	0.5 mL
R-PE Annexin V	496, 546, 565/578	29045-100 uL	20 assays
		29045-500 uL	100 assays
APC Annexin V	633, 640/660	29057-100 uL	20 assays
		29057-500 uL	100 assays
Biotin Annexin V, 50 ug/mL	N/A	29013	0.5 mL
5X Annexin V Binding Buffer	N/A	99902	15 mL

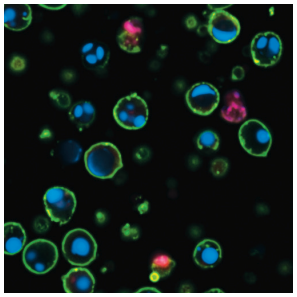

Figure 3. Jurkat cells stained using the Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus after apoptosis induction with staurosporine. Apoptotic cells stain with CF™488A Annexin V (green), necrotic/late apoptotic cells stain with EthDIII (red). All cells are stained with Hoechst (blue).

Apoptosis and Necrosis Detection Kits	Catalog number	Unit size
Dual Apoptosis Assay with NucView™ 488 and CF™594 Annexin V	30067	50 assays
Dual Apoptosis Assay with NucView™ 488 and CF™640R Annexin V	30073	50 assays
Apoptosis & Necrosis Quantitation Kit Plus	30065	50 assays
Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus	30066	50 assays
CF™488A Annexin V and 7-AAD Apoptosis Kit	30060	100 assays
CF™488A Annexin V and PI Apoptosis Kit	30061	100 assays

CF™ dye TUNEL kits and dUTP conjugates

TUNEL (terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick-end labeling) is highly selective for the detection of apoptotic cells, but not necrotic cells or cells with DNA strand breaks resulting from irradiation or drug treatment. In this assay, TdT enzyme catalyzes the addition of labeled dUTP to the 3' ends of cleaved DNA fragments. Fluorescent dye-conjugated dUTP can be used for direct detection of fragmented DNA by fluorescence microscopy or flow cytometry.

Biotium offers dUTP conjugated to a range of CF™ dye colors for fluorescent TUNEL labeling, as well as direct TUNEL kits with green fluorescent CF™488A, red fluorescent CF™594, and far-red fluorescent CF™640R. We also supply dUTP conjugated to classic fluorophores and biotin. Visit www.biotium.com to see our selection of CF™ dye conjugated streptavidin, as well as other nucleotide conjugates for probe labeling.

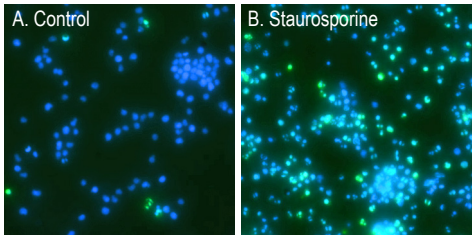

Figure 1. Jurkat cells labeled using the CF™488 TUNEL Assay Apoptosis Detection Kit after no treatment (A) or apoptosis induction with 1 uM staurosporine for 3 hours (B). Nuclei are counterstained with DAPI (blue).

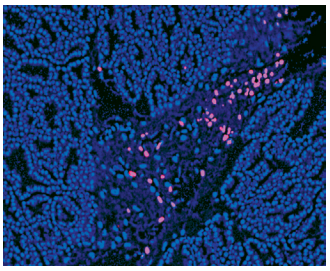

Figure 2. TUNEL staining of paraffin sections of rat mammary gland 5 days post-weaning (ApopTag® positive control slides, Millipore) using CF™594-dUTP (red). Nuclei are counterstained with DAPI (blue).

Cellular Senescence Assay Kit

Cellular senescence is a state of permanent cell cycle arrest that occurs in response to telomere shortening after many cycles of replication, cell confluence, or cell stress and damage. Senescent cells are characterized by enlarged flattened morphology and upregulation of lysosomal β-galactosidase. The Cellular Senescence Assay Kit uses the blue colorimetric β-galactosidase substrate X-gal to selectively stain senescent cells for detection by light microscopy.

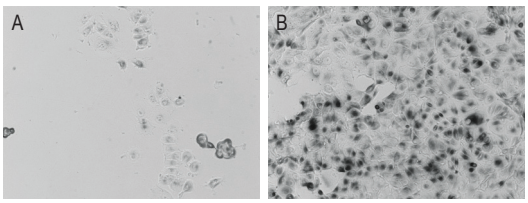

Figure 3. Detection of senescence-associated β-galactosidase activity in MCF-7 cells using the Cellular Senescence Assay Kit. A. Staining of sub-confluent proliferating cells. B. Staining of cells induced to enter senescence by high cell density and retinoic acid treatment.

PathoGreen™ Histofluorescent Stain

PathoGreen™ Histofluorescent Stain is an anionic green fluorescent dye functionally similar to Fluoro-Jade® dyes. These dyes stain degenerating neurons and their processes in fixed brain sections and cultured neurons. The dyes stain apoptotic and necrotic neurons after exposure to a variety of neurotoxic insults. The mechanism of neuronal staining by anionic fluorescent dyes has not been determined. It has been proposed that the negatively charged dyes bind to positively charged polyamines or other molecules specifically generated in dying neurons.

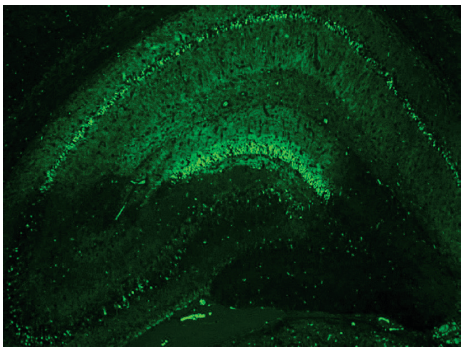

Figure 4. Degenerating neurons in a section of mouse hippocampus stained with PathoGreen™ Histofluorescent Stain.

TUNEL Assays and dUTP Conjugates	Ex/Em (nm)	Catalog no.	Unit Size
CF™488A TUNEL Assay Kit	490/515	30063	50 reactions
CF™594 TUNEL Assay Kit	593/614	30064	50 reactions
CF™640R TUNEL Assay Kit	642/662	30074	50 reactions
CF™405S-dUTP	404/431	40004	25 nmol
CF™488A-dUTP	490/515	40008	25 nmol
CF™543-dUTP	541/560	40002	25 nmol
CF™568-dUTP	562/583	40005	25 nmol
CF™594-dUTP	593/614	40006	25 nmol
CF™640R-dUTP	642/662	40007	25 nmol
CF™680R-dUTP	680/701	40003	25 nmol
Cyanine 555-dUTP	550/570	40064	25 nmol
Cyanine 647-dUTP	650/670	40065	25 nmol
DEAC-dUTP	426/480	40059	25 nmol
5-TAMRA-dUTP, 1 mM solution	553/577	40001	25 uL
Fluorescein-12-dUTP	494/521	40063	25 nmol
5-Bromo-dUTP, 10 mM solution	N/A	40025	25 uL
Biotin-11-dUTP, 1 mM solution	N/A	40029	50 uL
Biotin-11-dUTP, lyophilized	N/A	40029-1	50 ug
Biotin-16-dUTP, 1 mM solution	N/A	40022	50 uL
Biotin-16-dUTP, lyophilized	N/A	40022-1	50 ug
Biotin-20-dUTP, 1 mM solution	N/A	40030	50 uL
Biotin-20-dUTP, lyophilized	N/A	40030-1	50 ug

Histological Stains	Catalog number	Unit Size
PathoGreen™ Histofluorescent Stain, 1000X in water	80027-5mL	5 mL
	80027-50 mL	50 mL
Cellular Senescence Assay Kit	30031	100 assays

Live-or-Dye™ Fixable Viability Stains

Live-or-Dye™ for Fixable Staining of Dead Cells

- **Bright:** Biotium's superior dye technology allows maximum separation between live and dead cells.
- **Fixable:** No dye transfer between cells, compatible with permeabilization and immunostaining and storage of samples before analysis.
- **Multiple applications:** For flow cytometry or fluorescence microscopy.
- **Color selection**: Eight colors for multiplex flexibility.

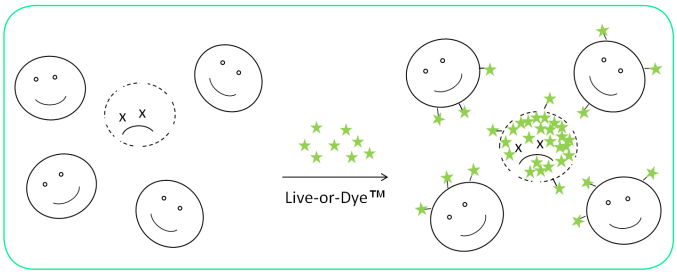

Figure 1. Principle of Live-or-Dye™ staining.

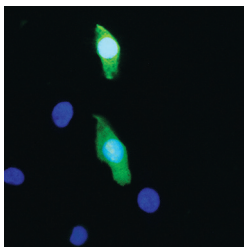

Figure 2. Live/dead cell imaging using Live-or-Dye™ Fixable Viability Stains. HeLa cells were treated with 15% ethanol for 10 minutes to kill a fraction of the cells. The cells were stained with Live-or-Dye™ 488/515 cell stain, followed by fixation with 4% formaldehyde, permeabilization with 0.1% Triton X-100, and counterstaining of nuclei with Hoechst dye. Live-or-Dye™ stained cells (green) can be clearly distinguished from unstained cells.

Live-or-Dye™ Fixable Viability Stains

Live-or-Dye™ Fixable Viability Staining Kits are designed for discrimination between live and dead cells by flow cytometry and microscopy. Viability stains are useful probes to include when analyzing cell surface protein expression by flow cytometry, because they allow intracellular fluorescence signal from dead cells with permeable plasma membranes to be excluded from analysis. Live-or-Dye™ Fixable Viability Stains are cell membrane impermeable amine-reactive dyes. The dyes enter dead cells that have compromised membrane integrity and covalently label free amines on intracellular proteins. On live cells, the dyes can potentially label surface proteins, but these are much less abundant than intracellular proteins, resulting in minimal labeling (Fig. 1-3). Live-or-Dye™ Fixable Viability Staining Kits can also be used to discriminate live from dead cells by microscopy (Fig. 3).

Live-or-Dye™ labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells. Biotium offers a selection of eight different Live-or-Dye™ viability stains spanning the fluorescence spectrum, for maximal flexibility in multi-color analysis.

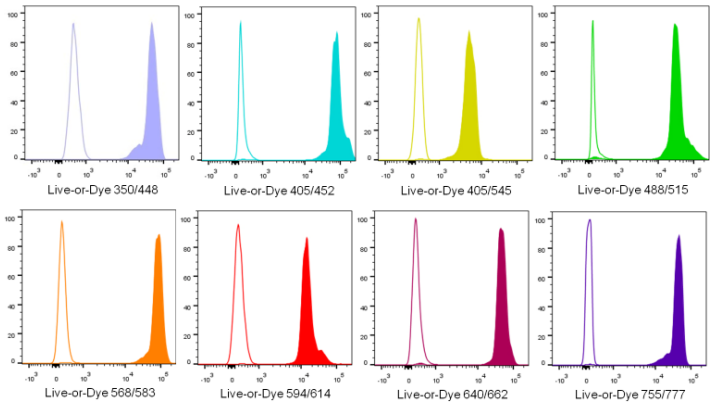

Figure 3. Discrimination of live and dead Jurkat cells by flow cytometry using Live-or-Dye™ Fixable Viability Stains. Heat killed cells (solid peaks) showed much higher fluorescence intensity compared to live cells (white peaks), allowing the two populations to be clearly distinguished.

Live-or-Dye™ Fixable Viability Staining Kits	Catalog No. (200 reactions)	Catalog No. (50 reaction trial size)	Laser line	Emission filter	Abs/Em maxima	Validated applications (FC=flow cytometry; M=microscopy)
Live-or-Dye™ 350/448	32002	32002-T	355 nm	DAPI or Violet	347/448 nm	FC
Live-or-Dye™ 405/452	32003	32003-T	405 nm	Pacific Blue	408/452 nm	FC
Live-or-Dye™ 405/545	32009	32009-T	405 nm	AmCyan	395/545 nm	FC
Live-or-Dye™ 488/515	32004	32004-T	488 nm	FITC	490/515 nm	FC, M
Live-or-Dye™ 568/583	32005	32005-T	488 or 561 nm	PE	562/583 nm	FC, M
Live-or-Dye™ 594/614	32006	32006-T	488 or 561 nm	PE-Texas Red®	561/624 nm	FC, M
Live-or-Dye™ 640/662	32007	32007-T	633 or 640 nm	APC	642/662 nm	FC, M
Live-or-Dye™ 750/777	32008	32008-T	633 or 640 nm	APC-Cy7	755/777 nm	FC

Texas Red is a registered trademark of Life Technologies.

ViaFluor™ Cell Proliferation Kits for Flow Cytometry

ViaFluor™ Cell Proliferation Dyes diffuse passively into cells and covalently label intracellular proteins, resulting in long term cell labeling. They are non-fluorescent until they are hydrolyzed by intracellular esterases. The dyes then react with intracellular amines forming fluorescent conjugates that are retained in the cell. Immediately after staining, a single, bright fluorescent population will be detected by flow cytometry. With each cell division, daughter cells inherit roughly half of the fluorescent label, allowing the number of cell divisions that occur after labeling to be detected by the appearance of successively dimmer fluorescent peaks on a flow cytometry histogram compared to cells analyzed immediately after staining. Staining is formaldehyde fixable. Cell proliferation assay kits contain ten single use dye vials, anhydrous DMSO for preparing dye stock solutions, and a detailed labeling protocol.

ViaFluor™405-SE Cell Proliferation Dye is excitable by the 405 nm violet laser with a fluorescence emission maximum at 452 nm. The dye can be analyzed in the violet channel by flow cytometry, freeing other channels for multi-color fluorescence assays.

ViaFluor™ CFSE Cell Proliferation Dye (also known as CFDA SE) is hydrolyzed in cells to release green fluorescent carboxyfluorescein, for detection in the FITC channel. CFDA SE (CFSE) is also as a stand-alone compound.

ViaFluor™568-SE Cell Proliferation Dye (Ex/Em 570/606 nm) has red fluorescence emission that is optimally excited by green laser lines (532-568 nm) for detection in the Texas Red® channel, but also can be detected in the PE-Texas Red® channel using 488 nm excitation.

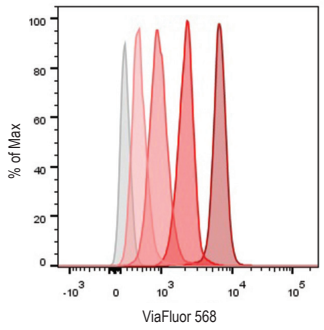

Figure 4. ViaFluor™ Cell Proliferation dyes stably label cells and can be used to track cell divisions over time, in vivo or in culture. Jurkat cells were treated with ViaFluor™ 568 and then maintained in cell culture for various amounts of time. As cells divide, each daughter cell receives approximately half of the dye contained in the parent. Peaks appear from right to left in progressively lighter shades of red for cells cultured 0 days (darkest red), 1 day, 2 days, or 3 days (lightest red) after staining. The peak for unstained cells is shown in gray.

Viability/Proliferation Assays	Catalog no.	Unit Size
ViaFluor™405-SE Cell Proliferation Kit	30068	1 kit
ViaFluor™ CFSE Cell Proliferation Kit	30050	1 kit
ViaFluor™568-SE Cell Proliferation Kit	30080	1 kit
CFDA SE, 5 and 6 isomers	90041	25 mg
Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells	30002-T	150 assays
	30002	300 assays
Calcein AM Cell Viability Assay Kit	30026	1000 assays
Resazurin Cell Viability Assay Kit	30025-1	25 mL (2500 assays)
	30025	100 mL (10,000 assays)
MTT Cell Viability Assay Kit	30006	1000 assays
XTT Cell Viability Assay Kit	30007	1000 assays
ATP-Glo™ Bioluminometric Cell Viability Assay Kit	30020-T	50 assays
	30020-1	200 assays
	30020-2	1000 assays

Calcein-AM Cell Viability Assay

Calcein-AM is a non-fluorescent, membrane permeable compound. Esterase activity in the cytoplasm of viable cells converts calcein-AM to the green fluorescent, membrane-impermeant compound calcein, which is retained in viable cells with intact plasma membranes. The Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells pairs calcein-AM with the vital dye Ethidium Homodimer III for quantitation of live and dead cells.

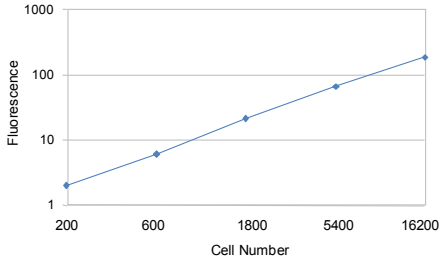

Figure 1. Quantitation of HeLa cell numbers using the Calcein AM Cell Viability Assay Kit. Cells were plated in 96-wells 24 hours before assay.

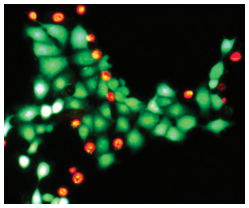

Figure 2. Live and dead HeLa cells stained with the Viability/Cytotoxicity Assay for Animal Live & Dead Cells. Live cells are stained green, dead cells are stained red.

ATP-Glo™ Bioluminometric Cell Viability Assay

This assay takes advantage of the ATP-dependent oxidation of D-Luciferin by Firefly luciferase and the resulting production of light in order to assess the amount of ATP in a cell culture, which is proportional to the number of viable cells. The ATP-Glo™ kit can be used to detect as little as a single cell or 0.01 picomole of ATP, with signal linearity for ATP detection within 6 orders of magnitude. This is a flash-type assay designed for detection using a single sample luminometer or a luminometer with an injector in 96-well plate format. The luminescent signal is stable for up to one minute.

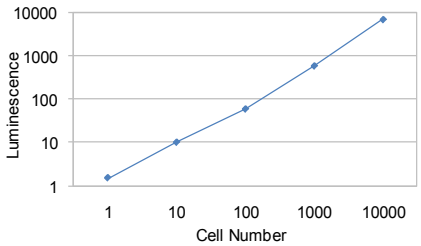

Figure 3. Quantitation of 10-fold serial dilutions of Jurkat cells in suspension using ATP-Glo™ Bioluminetric Cell Viability Assay using a single-sample luminometer.

Resazurin, MTT, and XTT Viability Assays

MTT, XTT, and resazurin (alamarBlue®) are reduced by mitochondrial metabolic activity to yield colored or fluorescent products, and thus are useful for assaying cell viability and quantitating cell number. MTT and XTT are reduced to colored formazin salts that can be measured by absorbance. MTT generates an insoluble formazin salt, requiring cell lysis before the absorbance can be measured, while XTT does not require cell lysis for measurement. Resazurin is a non-fluorescent blue dye that is reduced to the pink fluorescent compound resorufin, which can be measured by fluorescence or absorbance.

alamarBlue is a registered trademark of Morphosys UK Ltd.

Vital Dyes, Apoptosis Inducers, and Accessory Products

Vital dyes

Ethidium Homodimer III is a novel membrane-impermeant red nucleic acid dye developed at Biotium that is 70% brighter than ethidium homodimer I, for selective staining of dead cells.

RedDot™1 and RedDot™2 are novel far red nuclear stains developed at Biotium. RedDot™1 is a live cell stain, which also can be used for cell cycle analysis based on DNA content. RedDot™2 selectively stains cells with compromised membrane integrity, and also can be used for nuclear-specific counterstaining of fixed and permeabilized cells or tissue sections (Figure. 1).

Biotium also offers a selection of classic fluorescent nucleic acid stains such as propidium iodide, Hoechst dyes, and DAPI. Please visit www.biotium.com for more information.

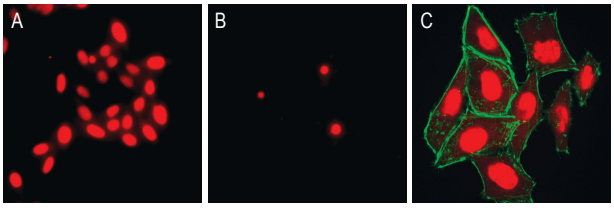

Figure 1. A. Nuclear staining of live HeLa cells with RedDot™1. B. Selective staining of dead HeLa cells with RedDot™2. C. Fixed and permeabilized HeLa cells stained with RedDot™2. Actin filaments are stained green with CF™488A phalloidin.

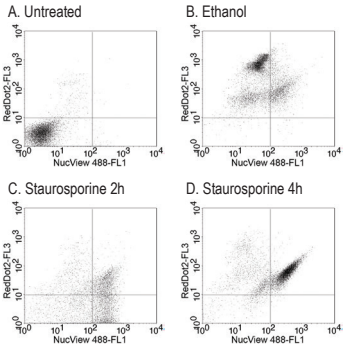

Figure 2. Flow cytometry analysis of Jurkat cells left untreated (A), treated with 10% ethanol for 90 minutes to induce necrosis (B), or treated with 1 uM staurosporine for 2 hours (C) or 4 hours (D) to induce apoptosis. NucView™ 488 was detected in the FL1 channel and RedDot™2 in the FL3 channel of a BD FACSCalibur flow cytometer. Necrotic cells stain high with RedDot™2 and low with NucView™ 488. Early apoptotic cells stain high with NucView™ 488 and low with RedDot™2, while late apoptotic cells stain high with both probes. See pp. 3-4 for more information on NucView™ substrates.

Chemical inducers of apoptosis

Staurosporine is a broad range protein kinase inhibitor that induces apoptosis in cultured cells. We also offer ionomycin, a calcium ionophore that has been shown to induce apoptosis through calpain activation.

Cell biology accessory products

Biotium offers unique accessory products for cell biology research. Also see the product information table below for convenient buffers and accessories.

EverBrite™ Mounting Media provide superior protection against photobleaching, and are compatible with cyanine-based dyes like Cy® dyes; available in wet-set and hard-set formats, and with DAPI.

TrueBlack™ Lipofuscin Autofluorescence Quencher suppresses lipofuscin autofluorescence, allowing detection of specific immunofluorescence signal in human and aged animal tissues. TrueBlack™ causes minimal increase in red background fluorescence compared to conventional Sudan Black B treatment (Fig. 3).

CoverGrip™ Coverslip Sealant is a unique alternative for sealing wet-set coverslips without the use of nail polish.

AccuEasy™ Flow Cytometry Kit allows staining and recovery of adherent cells for flow cytometry analysis without the use of trypsin, preserving cell surface epitopes. We also offer **Mini Cell Scrapers** for collecting cells from 24-well, 48-well, and 96-well microplates.

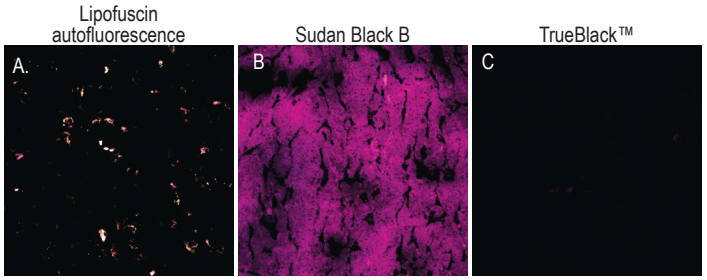

Figure 3. A. Lipofuscin autofluorescence in methanol-fixed adult human brain sections appears as fluorescent granules in all fluorescence channels, appearing yellow/white in the merged image above. B. Sudan Black B treatment masks lipofuscin autofluorescence, but introduces background in the red and far-red channels. C. TrueBlack™ masks lipofuscin with much less increase in fluorescence background. Samples were imaged at the same gain settings on a Zeiss LSM 700 confocal microscope in the FITC (green), Cy3 (red), and Cy5 (far-red) channels, merged images are shown.

Vital Dyes	Catalog no.	Unit Size
RedDot™1, 200X in water	40060-T	25 uL
	40060	250 uL
	40060-1	1 mL
RedDot™2, 200X in DMSO	40061-T	25 uL
	40061	250 uL
	40061-1	1 mL
NucView™ 488 and RedDot™2 Apoptosis & Necrosis Kit	30072	100 assays
Ethidium Homodimer III	40050	1 mg
Ethidium Homodimer III, 1 mM in DMSO	40051	200 uL

Apoptosis Inducers	Catalog no.	Unit Size
Staurosporine	00025	100 ug
Ionomycin, calcium salt	59007	1 mg

Accessory Products	Catalog no.	Unit Size
EverBrite™ Mounting Medium	23001	10 mL
EverBrite™ Mounting Medium with DAPI	23002	10 mL
EverBrite™ Hardset Mounting Medium	23003	10 mL
EverBrite™ Hardset Mounting Medium with DAPI	23004	10 mL
CoverGrip™ Coverslip Sealant	23005	15 mL
TrueBlack™ Lipofuscin Autofluorescence Quencher	23007	1 mL
AccuEasy Flow Cytometry Kit	30069	1 kit
Mini Cell Scrapers	22003	Pack of 200
Flow Cytometry Fixation/Permeabilization Kit	23006	50 tests
Mini Super^{HT} Pap Pen	22005	1 pen
Super^{HT} Pap Pen	22006	1 pen
Fixation Buffer	22015	100 mL
Permeabilization Buffer	22016	100 mL
Permeabilization and Blocking Buffer (5X)	22017	100 mL
Fish Gelatin Blocking Agent	22010	100 mL

PMA™ and PMAxx™ Viability PCR and Other Bacterial Viability Assays

PMA™ and PMAxx for selective detection of live cells by qPCR

PMA™ is a membrane impermeable, photo-reactive DNA-binding dye. When a bacterial sample is treated with PMA™ and light, only dead bacteria are susceptible to DNA modification that prevents amplification by PCR (Figure 1). Thus, subsequent analysis by qPCR permits selective detection of live cell DNA (Figure 2). PMA™ has been used in a variety of bacterial strains from diverse sources, as well some strains of yeast and viruses. Visit www.biotium.com to download a list of references.

PMAxx™ is Biotium's next-generation photoreactive viability dye. It has been optimized for less penetration into live cells and higher activity in dead cells, for better live/dead discrimination. Visit www.biotium.com to learn more.

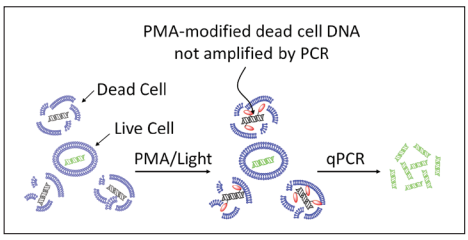

Figure 1. Principle of selective qPCR of live bacteria after treatment with PMA™ and light.

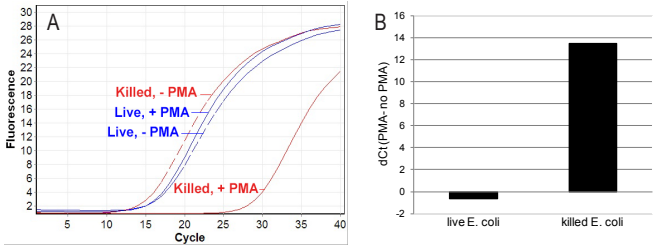

Figure 2. Effect of PMA™ on quantitative PCR of live and heat-inactivated E. coli. A. Quantitation analysis of real-time PCR performed on live and dead E. coli treated with PMA™ using primers against a region of the 16S rRNA. B. The ΔCt of live and killed E. coli with and without PMA™ treatment. The Ct value of sample without PMA™ was subtracted from the corresponding sample with PMA™ cross-linking (Ct with PMA - Ct without PMA).

NEW! PMA™ Enhancer for Gram-Negative Bacteria

Under mild killing conditions such as low heat treatment, dead bacteria may retain intact membranes that have lower permeability to PMA™ compared to bacteria subjected to harsh killing methods like high heat treatment, for example. This could result in an overestimate of live bacteria. Biotium has developed PMA Enhancer for use with Gram-negative bacteria that can greatly improve live/dead discrimination (Fig. 3).

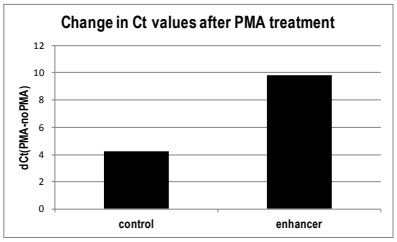

Figure 3. PMA™ plus Enhancer for quantitation of viable bacteria by Real-time PCR. Mildly heat-killed E. coli were treated with PMA™ with or without Enhancer, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 377-bp fragment of E. coli DNA. Dead cells treated with PMA™ + Enhancer showed improved discrimination between live and dead cells compared to dead cells treated with PMA™ alone.

Biotium also offers EvaGreen® dye-based qPCR master mixes and probe PCR master mixes; visit www.biotium.com for more information.

NEW! PMA™ Real-Time PCR Bacterial Viability Kits

Convenient kits with PMA™, PMA™ Enhancer (Gram-negative strains only), PCR primers, and Fast EvaGreen® qPCR Master Mix, for viability PCR of specific strains. Don't see a kit for your favorite bacteria? Let us know at techsupport@biotium.com.

Kits available for:

- *Salmonella enterica*
- *Staphylococcus aureus*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- *Escherichia coli*
- *Escherichia coli* O157:H7
- *Mycobacterium tuberculosis*
- *Listeria monocytogenes*

PMA-Lite™ LED Photolysis Device

- Designed for efficient activation of PMA™, EMA or other similar azido dyes
- Provides even illumination to up to 18 1.5-2 mL-sized vials
- Internal fan ensures a temperature of <37°C
- Four timer settings for 10, 15, 20 or 30 minutes of illumination
- Long-lasting LEDs with 465-475 nm emission

Figure 4. PMA-Lite™ LED Photolysis Device.

Viability/Cytotoxicity Assay kit for Bacteria

In this kit, membrane permeable green fluorescent dye DMAO stains all bacteria, and ethidium homodimer III stains dead cells with red fluorescence. For fluorescence microscopy, plate reader, or flow cytometry.

Bacterial Viability and Gram Stain Kit

CF™488A wheat germ agglutinin stains gram-positive cells green, while DAPI stains all cells blue, and ethidium homodimer III stains dead cells red. For fluorescence microscopy, plate reader, or flow cytometry.

Bacterial Viability Reagents and Kits	Catalog number	Unit Size
PMA™ dye	40013	1 mg
PMA™ dye, 20 mM in water	40019	100 uL
PMAxx™ dye, 20 mM in dH2O	40069	100 uL
PMA Enhancer for Gram-Negative Bacteria	31038	16 mL
PMA PCR Bacterial Viability Kit - *Salmonella enterica*	31033	200 assays
PMA PCR Bacterial Viability Kit - *M. tuberculosisis*	31034	200 assays
PMA PCR Bacterial Viability Kit - *Staph. aureus*	31035	200 assays
PMA PCR Bacterial Viability Kit - *Staph. aureus* (mecA)	31036	200 assays
PMA PCR Bacterial Viability Kit - *E. coli*	31050	200 assays
PMA PCR Bacterial Viability Kit - *E. coli* O157:H7	31037	200 assays
PMA PCR Bacterial Viability Kit - *Listeria monocytogenes*	31051	200 assays
PMA-Lite™ LED Photolysis Device	E90002	Each
Ethidium monoazide, bromide (EMA dye)	40015	5 mg
Viability/Cytotoxicity Assay kit for Bacteria	30027	100-1000 assays
Bacterial Viability and Gram Stain Kit	32001	200 assays

Glowing Products for Science™

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order@biotium.com

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