Cell Viability and Cell Death

Biotium

NucView® Caspase-3 Substrates

Fluorescent caspase-3 detection in living cells

NucView® caspase-3 substrates are novel fluorescent probes that allow detection of caspase-3/7 activity in intact cells in real-time. In contrast to other fluorogenic caspase substrates or fluorescent caspase inhibitor based (FLICA) assays, NucView® substrates can be used to detect caspase-3/7 activity in cells without inhibiting apoptosis progression.

NucView® is made by attaching a nucleic acid binding dye to the caspase-3/7 substrate peptide sequence DEVD. This uncleaved substrate dye is unable to bind to DNA and remains non-fluorescent. Once the substrate is cleaved by caspase-3/7 in apoptotic cells, it releases the high-affinity fluorescent DNA dye, which stains the cell nucleus with bright and stable fluorescence signal.

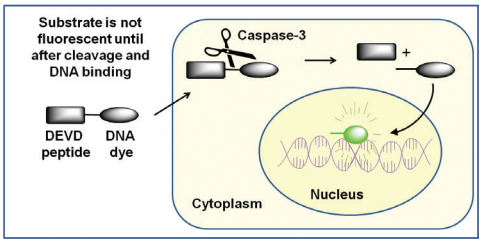
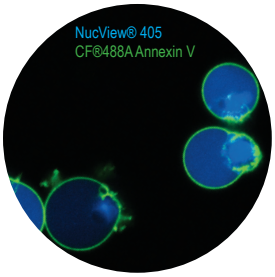
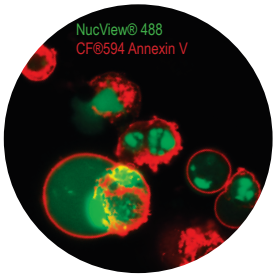


Figure 1. Schematic showing the principle of intracellular caspase-3/7 detection using NucView® caspase-3 substrates.

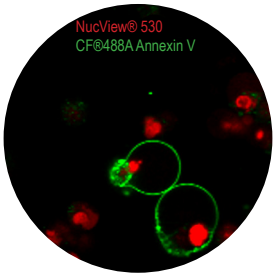
NucView® 405





NucView® 488

NucView® 530



Proven technology

NucView® caspase detection technology has been extensively tested. NucView® 488 Substrate has been:

- Published in over **200 scientific papers**
- Validated in more than **70 different cultured cell lines**
- Validated in more than **30 different primary cell types**

NucView® features

- Real-time monitoring of caspase-3/7 activity
- Rapid, no-wash assay
- Available in 3 colors
- For flow cytometry, microscopy or microplate reader
- Formaldehyde fixable

NucView® Substrates

Cat. #	Size	Description
10402-T	10 uL	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10402	100 uL	
10403-T	10 uL	NucView® 488 Caspase-3 Substrate, 1 mM in PBS
10403	100 uL	
10405-T	10 uL	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10405	100 uL	
10407-T	10 uL	NucView® 405 Caspase-3 Substrate, 1 mM in PBS
10407	100 uL	
10406-T	10 uL	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO
10406	100 uL	
10408-T	10 uL	NucView® 530 Caspase-3 Substrate, 1 mM in PBS
10408	100 uL	

NucView® kits and other caspase assays

NucView® combination staining kits

Biotium also offers kits containing the NucView® 488 substrate together with other types of apoptosis and viability dyes for convenient multi-parameter experiments.

- Dual Apoptosis Kit: NucView® 488 + Annexin V labeled with red or far-red dyes for co-detection of two apoptotic events, caspase cleavage and phosphatidylserine (PS) translocation. For more Annexin V conjugates see p. 4.
- Dual Apoptosis Kit: NucView® 488 + MitoView™ 633 for co-detection of two apoptotic events, caspase cleavage and loss of mitochondrial membrane potential. For more information on MitoView™ see p. 6.
- Apoptosis/Necrosis Kit: NucView® 488 + RedDot™2 for concurrent measurement of caspase cleavage (apoptosis) and loss of membrane integrity (necrosis).

Additional caspase substrates

In addition to our patented NucView® technology for detecting caspase-3 activity in live cells, Biotium also offers rhodamine 110 (R110)-based assay kits for fluorescence- or absorbance-based detection of caspase-3 or caspase-8 activity in cell lysates. The HTS versions of the R110-based homogenous caspase-3 and caspase-8 assay kits are optimized for high throughput screening by fluorescence microplate reader.

Biotium also offers a coumarin (AMC)-based blue fluorogenic substrate (Ac-DEVD-AMC) for measuring caspase-3 activity in cell lysates by fluorescence microplate reader.

Caspase inhibitor

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

Apoptosis inducers

Staurosporine is a broad range protein kinase inhibitor that induces apoptosis in cultured cells. It is useful as a positive control for many apoptosis assays. We also offer ionomycin, a calcium ionophore that has been shown to induce apoptosis through calpain activation.

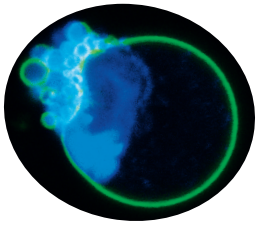


Figure 2. Apoptotic HeLa cell stained with CF®488A Annexin V (green) and NucView® 405 (cyan). See p. 4 for more information on Annexin V conjugates.

NucView® Combination Kits and Other Caspase Substrates and Inhibitors

Cat. #	Size	Description
30067	50 assays	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®594 Annexin V
30073	50 assays	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®640R Annexin V
30062	100 assays	NucView® 488 and MitoView™ 633 Apoptosis Assay Kit
30072	100 assays	NucView® 488 and RedDot™2 Apoptosis & Necrosis Kit
30029-T	25 assays	NucView® 488 Caspase-3 Assay Kit for Live Cells
30029	100 assays	
30008-1	25 assays	Caspase-3 DEVD-R110 Fluorometric & Colorimetric Assay Kit
30008-2	100 assays	
30009-1	10 assays	Caspase-3 DEVD-R110 Fluorometric HTS Assay
30009-2	100 assays	
30009-3	1000 assays	
30011-1	25 assays	Caspase-8 IETD-R110 Fluorometric & Colorimetric Assay Kit
30011-2	100 assays	
30012-1	10 assays	Caspase-8 IETD-R110 Fluorometric HTS Assay
30012-2	100 assays	
30012-3	1000 assays	
10404-1	1 mg	Ac-DEVD-CHO Caspase-3 Inhibitor
10404	5 mg	
10202	5 mg	Ac-DEVD-AMC
00025	100 ug	Staurosporine
59007	1 mg	Ionomycin, calcium salt

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). 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.

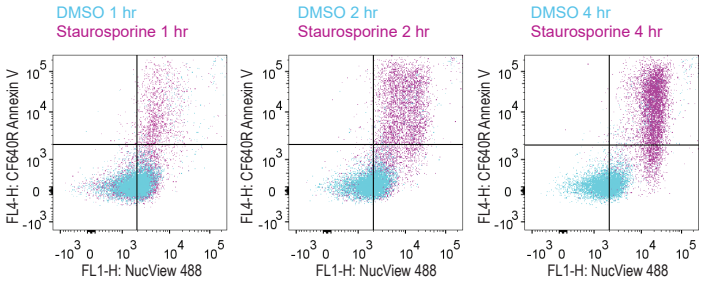


Figure 1. Jurkat cells were treated with staurosporine to induce apoptosis (pink), or with DMSO as a negative control (blue) for the times indicated, then stained for 15 minutes at room temperature with NucView® 488 Caspase-3 Substrate (FL1-H, x-axis) and CF®640R Annexin V (FL4-H, y-axis) in cell culture medium prior to analysis using a BD LSRII flow cytometer. See pp. 2-3 for more information on NucView® caspase-3 substrates.

CF®488A Annexin V Dual Apoptosis & Necrosis Assay Kits

Biotium offers several staining kits that allow concurrent identification of late apoptotic and membrane-compromised necrotic cells by fluorescence microscopy or flow cytometry. These dual staining kits all include green fluorescent CF®488A Annexin V paired with a dead cell-specific nucleic acid dye: either red fluorescent Ethidium Homodimer III (EthD-III), red fluorescent propidium iodide (PI), or far-red fluorescent 7-AAD. EthD-III is 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 (Fig. 2).

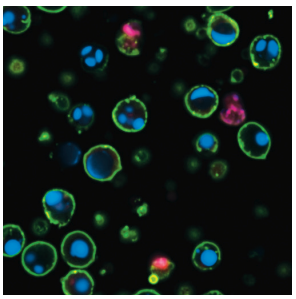


Figure 2. Jurkat cells stained using the Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus after apoptosis induction with staurosporine. Apoptotic cells stain with CF®488A Annexin V (green), necrotic/late apoptotic cells stain with EthDIII (red). All cells are stained with Hoechst (blue).

Annexin V Conjugates

Annexin V Conjugate	Ex/Em (nm)	Cat. #	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®660R Annexin V, 50 ug/mL	663/682	29069	0.5 mL
CF®680R Annexin V, lyophilized	680/701	29070	25 ug
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
CF®800 Annexin V, lyophilized	797/816	29078	25 ug
FITC Annexin V, 50 ug/mL	490/525	29001	0.5 mL
Texas Red Annexin V, 50 ug/mL	583/603	29002	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

Apoptosis and Necrosis Combination Kits

Kit Name/Description	Cat. #	Size
Apoptosis & Necrosis Quantitation Kit Plus with CF®488A Annexin V and EthD-III	30065	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
Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus with CF®488A Annexin V, EthD-III and Hoechst	30066	50 assays

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 p. 2 for more information on NucView® substrates).

Dual Apoptosis Kits

Kit Name	Cat. #	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

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.

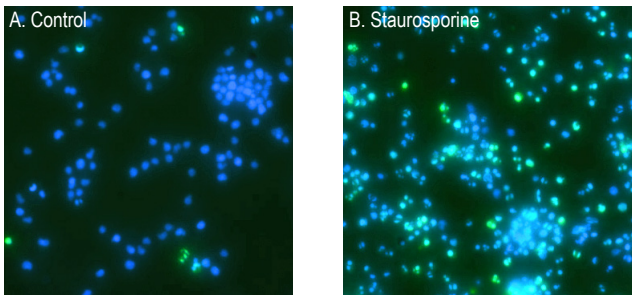


Figure 1. Jurkat cells labeled using the CF®488A TUNEL Assay Apoptosis Detection Kit after no treatment (A) or apoptosis induction with 1 uM staurosporine for 3 hours (B). Nuclei are counterstained with DAPI (blue).

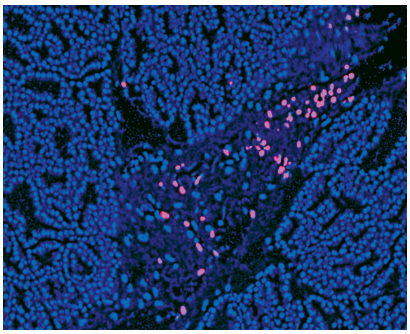


Figure 2. TUNEL staining of paraffin sections of rat mammary gland five days post-weaning (ApopTag® positive control slides, MilliporeSigma) using CF®594-dUTP (red). Nuclei are stained with DAPI (blue).

TUNEL Assays and dUTP Conjugates

Product Name	Ex/Em (nm)	Cat. #	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-T	5 nmol
		40004	25 nmol
CF®405M-dUTP	408/452	40100-T	5 nmol
		40100	25 nmol
CF®488A-dUTP	490/515	40100-T	5 nmol
		40008	25 nmol
CF®543-dUTP	541/560	40008-T	5 nmol
		40002	25 nmol
CF®568-dUTP	562/583	40002-T	5 nmol
		40005	25 nmol
CF®594-dUTP	593/614	40005-T	5 nmol
		40006	25 nmol
CF®640R-dUTP	642/662	40006-T	5 nmol
		40007	25 nmol
CF®680R-dUTP	680/701	40007-T	5 nmol
		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-AA-dUTP, 10 mM	N/A	40020	100 uL
5-AA-dUTP, lyophilzed	N/A	40020-1	1 mg
Digoxigenin-dUTP	N/A	40078	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

Mitochondrial Membrane Potential Dyes

MitoView™ Dyes

Loss of mitochondrial membrane potential is a hallmark for apoptosis. Biotium offers the MitoView™ 633 dye for membrane potential-sensitive staining of mitochondria by microscopy or flow cytometry. We also offer MitoView™ Blue, which changes localization upon mitochondrial depolarization, and MitoView™ Green, a membrane-potential independent mitochondrial dye that can be used to image mitochondria following mitochondrial depolarization, or after fixation.

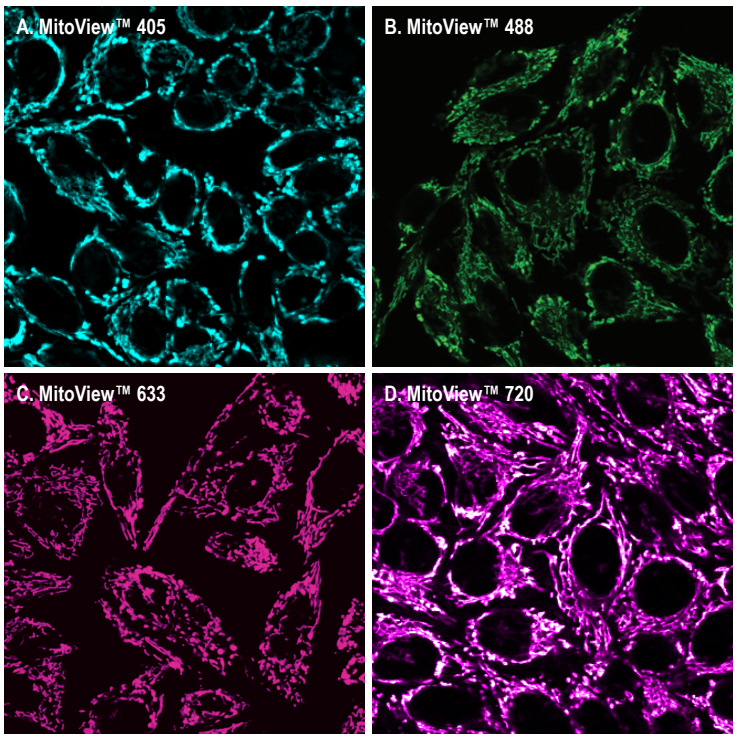


Figure 4. HeLa cells stained with A. MitoView™ Blue, B. MitoView™ Green, C. MitoView™ 633, or D. MitoView™ 720.

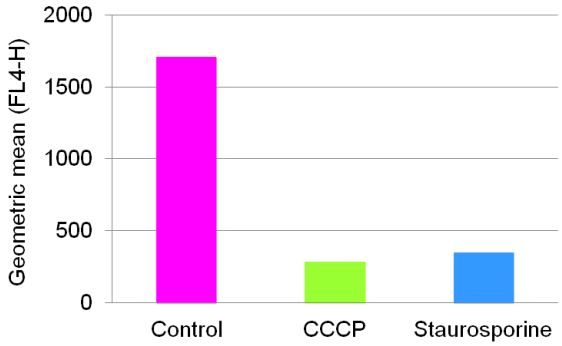


Figure 5. Flow cytometry analysis of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in decreased MitoView™ 633 staining.

MitoView™ Dyes

Cat. #	Product	Ex/Em (nm)	Potential-dependent?	Unit Size
70070-T	MitoView™ 405	398/440	Partial†	50 ug
70070				20 x 50 ug
70054-T	MitoView™ Green	490/523	No	50 ug
70054				20 x 50 ug
70055-T	MitoView™ 633	622/648*	Yes	50 ug
70055				20 x 50 ug
70068-T	MitoView™ 720	720/758 nm**	Partial†	50 ug
70068				20 x 50 ug

*MitoView™ 633 also has visible red fluorescence in the Cy®3/rhodamine channel. It is not recommended for imaging with other visible red probes.

**While optimal for Cy®7 settings, MitoView™ 720 is bright enough to be imaged in the Cy®5 channel, and can be combined with visible red fluorescent probes.

†Dyes with partial mitochondrial membrane potential dependence localize to the cytoplasm after mitochondrial depolarization, but still retain fluorescence.

Mitochondrial Membrane Potential and Cellular Glutathione

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, including TMRE, TMRM, and DiIC1(5), in a variety of fluorescent colors.

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.

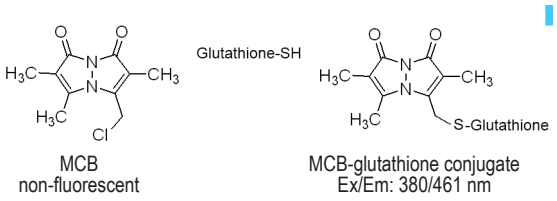


Figure 6. MCB glutathione assay principle.

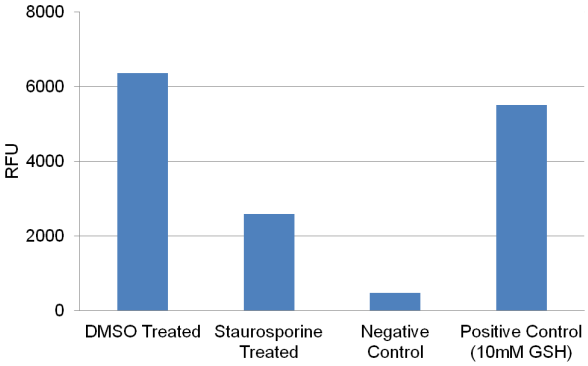


Figure 7. Jurkat cells were treated with DMSO (Control) or induced to undergo apoptosis by treatment with 1 uM staurosporine for 5 hours. Glutathione levels were measured using the MCB Glutathione Detection Kit by fluorescence microplate reader.

Other Mitochondrial Dyes

Dye	Color	Ex/Em	Mitochondrial Membrane Potential Dependent?	Cat. #	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

Kit Name and Components	Color	Ex/Em	Assay	Cat. #	Size
NucView® 488 and MitoView™ 633 Apoptosis Kit	Green/ Red	500/530 nm (caspase-3) 622/648 nm (polarized mitochondria)	Two color detection of caspase-3 activity and mitochondrial potential	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

Live-or-Dye™ Fixable Viability Stains

Live-or-Dye™

Live-or-Dye™ Fixable Viability Staining Kits are designed for discrimination between live and dead cells by flow cytometry and microscopy. Dead cell stains are useful probes to include when analyzing cell surface protein expression by flow cytometry (Fig. 2), 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; they enter dead cells that have compromised membrane integrity and covalently label free amines on intracellular proteins. Live-or-Dye™ Fixable Viability Staining Kits can also be used to discriminate live from dead cells by microscopy (Fig. 1A). Live-or-Dye™ labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

Live-or-Dye™ NucFix Red

Live-or-Dye NucFix™ Red is a unique, cell membrane impermeable dye that specifically stains the nuclei of dead cells (Fig. 1B). The dye is able to enter into dead cells that have compromised membrane integrity and covalently label the cell nucleus, allowing for clear differentiation of live and dead cells by either microscopy or flow cytometry. Unlike other commonly used nuclear stains such as propidium iodide or DRAQ®7, NucFix labeling is extremely stable, allowing the cells to be fixed and permeabilized without loss of fluorescence or dye transfer between cells.

Live-or-Dye™ Fixable Stains for Dead Cells

- **Affordable**: Lower cost than Thermo Fisher LIVE/DEAD® stains
- **Choice**: 8 bright colors across the spectrum
- **Specific**: No staining of live cells
- **Fixable**: No loss of brightness after fixation
- **Versatile**: For flow cytometry and microscopy

Live-or-Dye™ NucFix Red

- Unique, nuclear dead cell stain
- Fixable, unlike other commonly used nuclear stains such as Propidium Iodide or DRAQ®7

A. Live-or-Dye™ 488/515 B. Live-or-Dye™ NucFix Red

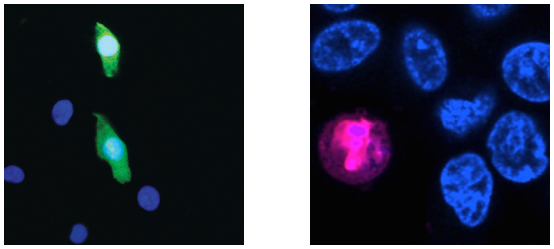


Figure 1. HeLa cells were treated with ethanol to kill a fraction of the cells. The cells were stained with A) Live-or-Dye™ 488/515 or B) Live-or-Dye™ NucFix Red. Nuclei were counterstained with Hoechst.

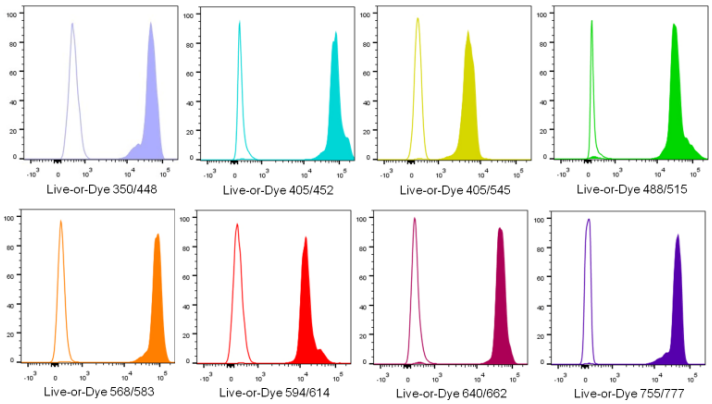


Figure 2. Discrimination of live and dead Jurkat cells by flow cytometry using Live-or-Dye™ Fixable Viability Stains. Heat killed cells (solid peaks) showed much higher fluorescence intensity compared to live cells (white peaks), allowing the two populations to be clearly distinguished.

Live-or-Dye™ Kits

Kit Name	Cat. # 200 Reactions	Cat. # 50 Reactions	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®	593/614 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
Live-or-Dye™ NucFix Red	32010	32010-T	488 or 532 nm	PE-Texas Red®	520/610 nm	FC, M

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 dead cell dye Ethidium Homodimer III for quantitation of live and dead cells.

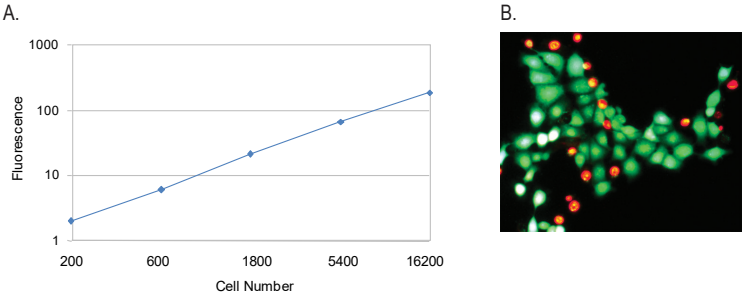


Figure 1. A. Quantitation of HeLa cell numbers in a 96-well assay plate using the Calcein AM Cell Viability Assay Kit. B. Live and dead HeLa cells stained with the Viability/Cytotoxicity Assay for Animal Live & Dead Cells. Live cells are stained green, dead cells are stained red.

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.

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.

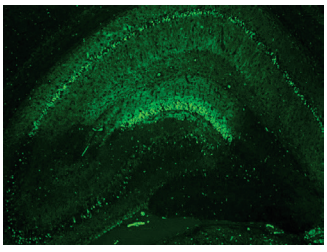


Figure 2. A section of mouse hippocampus stained with PathoGreen™ Histofluorescent Stain. Degenerating neurons are stained green.

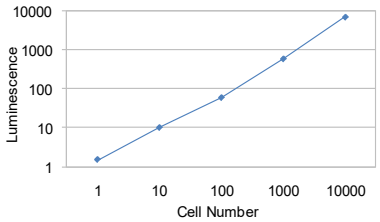


Figure 3. Quantitation of 10-fold serial dilutions of Jurkat cells in suspension using ATP-Glo™ Bioluminetric Cell Viability Assay using a single-sample luminometer.

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.

ViaFluor® SE Cell Proliferation Kits

ViaFluor® SE Cell Proliferation Kits diffuse passively into cells covalently label intracellular proteins throughout the cell. They can be used as cell-filling stains for imaging morphology, or to track cell division by dye dilution. With each cell division, daughter cells inherit roughly half of the fluorescent label, allowing the number of cell divisions to be detected by the appearance of successively dimmer fluorescent peaks on a flow cytometry histogram. Staining is formaldehyde fixable.

ViaFluor® CFSE is the classic cell proliferation dye, detected in the FITC channel. Biotium created ViaFluor® 488, a new improved green dye that is less toxic, less leaky and more fixable than CFSE. We also offer blue fluorescent ViaFluor® 405 for the violet laser. ViaFluor® 405 has improved brightness and less toxicity than CFSE.

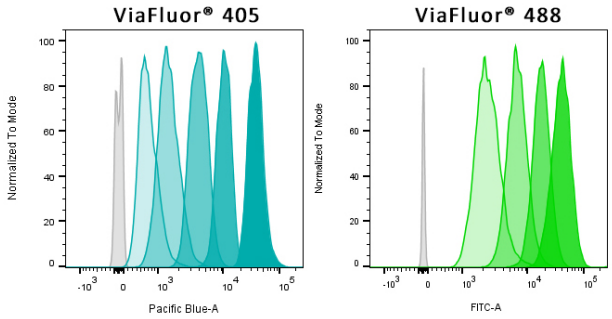


Figure 3. Cell division tracking in Jurkat cells over successive days. Cells were labeled with ViaFluor® 405 (left) or ViaFluor® 488 (right) on day 0, and analyzed by flow cytometry on each following day. Each successively dimmer peak represents one cell division. Unstained cells are in gray.

Cellular Viability Assays

Kit Name	Cat. #	Unit Size
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
PathoGreen™ Histofluorescent Stain, 1000X in water	80027-5mL	5 mL
	80027-50mL	50 mL
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
ViaFluor® 405 SE Cell Proliferation Kit	30068	1 kit
ViaFluor® 488 SE Cell Proliferation Kit	30086	1 kit
ViaFluor® CFSE Cell Proliferation Kit	30050	1 kit

PMAxx™ and PMA Dyes for Viability PCR

Viability PCR (v-PCR)

Viability PCR is a powerful technology for the sensitive and rapid detection of viable microorganisms. Unlike time-consuming culturing procedures, qPCR is a fast and sensitive method of detection. However, normal qPCR does not distinguish between live and dead cells. With v-PCR using PMAxx™ or PMA, you get the speed, sensitivity and specificity of PCR, plus quantifiable viability. And because no culturing is required, you can even detect viable but not culturable (VBNC) bacteria.

How does v-PCR work?

PMAxx™ and PMA are photoreactive dyes with high affinity for DNA. The dyes intercalate into dsDNA and form a covalent linkage upon exposure to intense visible light. PMAxx™ and PMA inhibit PCR amplification of modified DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases. Because PMAxx™ and PMA are cell membrane-impermeable, when a sample containing both live and dead bacteria is treated with dye, only dead bacteria with compromised cell membranes are susceptible to DNA modification (Fig. 1). In a real-time PCR reaction, dead cell DNA will show delayed amplification and higher Ct than live cells. In a mixed population, v-PCR permits quantitation of cell viability. The v-PCR technology can be applied not only to bacteria but to other cell types as well.

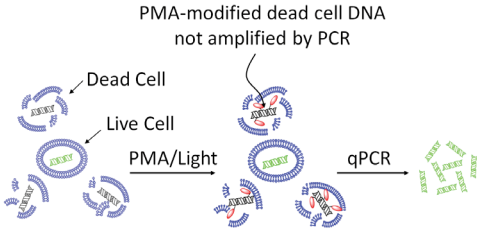


Figure 1. Mechanism of PMA and PMAxx™. The cell membrane-impermeable dyes PMA and PMAxx™ selectively and covalently modify DNA from dead bacteria with compromised membranes while leaving DNA from viable cells intact.

PMAxx™ vs PMA

Since Biotium developed PMA in 2006, it has been used extensively in many applications and in hundreds of publications. However there are cell types and conditions in which dead cell DNA inactivation by PMA is incomplete, which could lead to false positive results. After extensive testing, the scientists at Biotium have invented a new dye called PMAxx™ that has the same spectral properties and is even more effective than PMA at live/dead discrimination by viability PCR (Fig. 2).

v-PCR LED Photolysis Devices

PMA-Lite™:

- Holds up to 18 microcentrifuge tubes
- Bright, long-lasting blue LED lights
- Fan ensures temperatures lower than 37°C.

Glo-Plate™ Blue:

- Flat illumination surface fits microplates
- Bright, long-lasting blue LED lights
- Surface stays cool during light exposure.

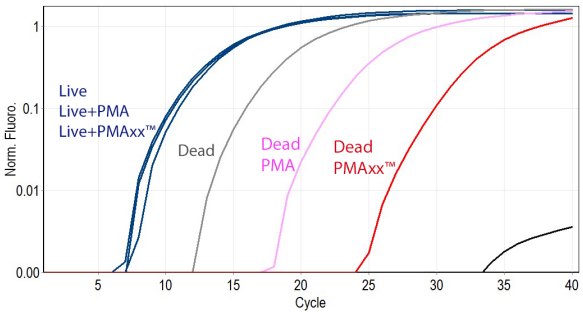


Figure 2. Live or heat-killed *Bacillus subtilis* were treated with PMA or PMAxx™, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 500-bp fragment of *B. subtilis* DNA. qPCR of dead cells treated with PMAxx™ showed a significant further delay (>7 Ct) compared to dead cells treated with PMA.

Strain-specific v-PCR kits available for:

- Salmonella enterica
- Escherichia coli
- Escherichia coli O157:H7
- Listeria monocytogenes
- Legionella pneumophila
- Mycobacterium tuberculosis
- Staphylococcus aureus
- Methicilin resistant Staphylococcus aureus (MRSA)

Ordering Information

Cat. #	Product Name	Unit Size
40069	PMAxx™ dye, 20 mM in dH_2O	100 uL
40013	PMA dye	1 mg
40019	PMA dye, 20 mM in dH_2O	100 uL
E90002	PMA-Lite™ LED Photolysis Device	1 device
E90004	Glo-Plate™ Blue	1 device
31038	PMA Enhancer for Gram-Negative Bacteria	16 mL
31033	Real-Time Bacterial Viability Kit-Salmonella (InvA)	200 assays
31034	Real-Time Bacterial Viability Kit-M. tuberculosis (groEL2)	200 assays
31035	Real-Time Bacterial Viability Kit-Staph. aureus (nuc)	200 assays
31036	Real-Time Bacterial Viability Kit-MRSA (mecA)	200 assays
31050	Real-Time Bacterial Viability Kit-E. coli (uidA)	200 assays
31037	Real-Time Bacterial Viability Kit-E. coli O157:H7 (Z3276)	200 assays
31051	Real-Time Bacterial Viability Kit-Listeria monocytogenes (hly)	200 assays
31053	Real-Time Bacterial Viability Kit-Legionella pneumophila (mip)	200 assays

Bacteria Viability Dyes and Kits

Live-or-Dye™ Fixable Viability Staining Kits utilize dead-cell-specific fixable dyes (Fig. 1). They are good for flow cytometry and microscopy and available in 9 bright, photostable colors. See p. 8 for more information on Live-or-Dye™ stains. CTC is a fluorescent dye that has been used to evaluate the respiratory activity in bacteria. Healthy cells will reduce CTC into an insoluble red product. The Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells features dual staining: DMAO for live cells, and EthD-III for dead cells (Fig. 2).

Combination Gram Stain and Viability Kits

It can be useful to distinguish live bacteria from dead, as well as Gram+ from Gram-, in the same sample. Our combination bacterial viability and fluorescent gram staining kits can help (Fig. 3). Our fluorescent gram stains utilize fluorescently-labeled wheat germ agglutinin (WGA) to selectively stain the cell walls of gram-positive bacteria.

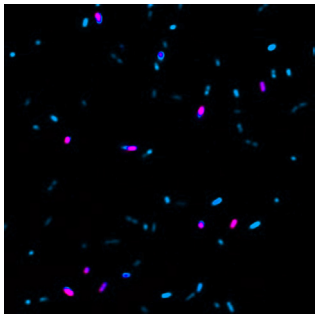


Figure 1. Live and heat-killed *E. coli* stained with Live-or-Dye™ 568/583 (red) and DAPI (blue).

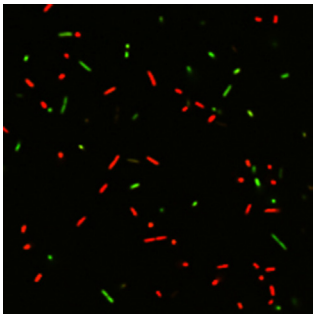


Figure 2. Live and heat-killed *E. coli* stained with DMAO, marking live cells (green) and EthD-III, marking dead cells (red).

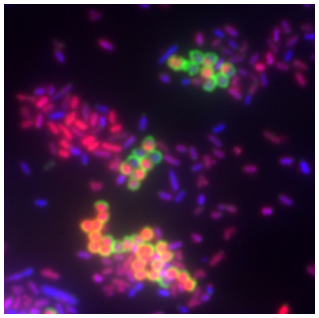


Figure 3. Bacterial Viability and Gram Stain Kit. CF®488A-WGA, EthD-III and DAPI.

Bacteria Viability Stains

Cat. #	Product	Description
10063	CTC (5-Cyano-2,3-ditolyl tetrazolium chloride)	Forms insoluble red product in respiring cells
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells	DMAO to stain all cells and EthD-III for dead cells
32001	Bacterial Viability and Gram Stain Kit	WGA for gram stain, EthD-III for dead cells, and DAPI for all cells

Yeast Viability Dyes and Kits

It is often useful to distinguish live yeast cells from dead, or identify cells that are metabolically active. Our selection of yeast viability dyes and kits can help.

- Live-or-Dye™ Fixable Viability Staining Kits: Fixable and dead-cell-specific. Good for flow cytometry and microscopy. Available in 9 bright, photostable colors. Note: the NucFix™ Red variation is not nucleus-specific in yeast. See p. 8 for more information on Live-or-Dye™ stains.
- ViaVac™ Red/Green: A vacuolar cell vitality dye. Passively diffuses into cells and gives a nonspecific green staining pattern. In metabolically active yeast, the dye is actively transported into the vacuole where it stains intravacuolar tubules bright red.

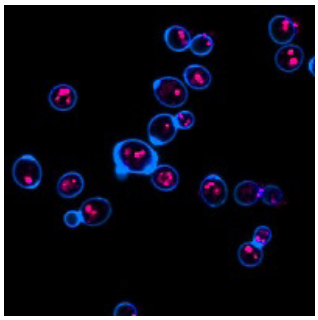


Figure 4. Yeast Vitality Staining Kit, ViaVac™ Red/Green (red, healthy vacuolar tubules) and Calcofluor White (blue, cell wall).

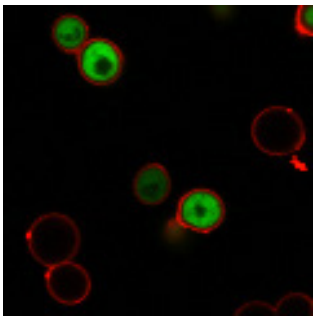


Figure 5. Yeast Viability Staining Kit, CF®-ConA (red, cell wall) and Live-or-Dye™ (green, dead cell cytoplasm).

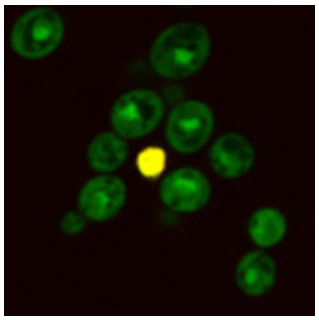


Figure 6. Yeast Fixable Live/Dead Staining Kit, Thiazole Orange (green, all cells) and Live-or-Dye™ 568/583 (red, dead cell cytoplasm). Overlapping signal appears yellow.

Yeast Viability Stains

Cat. #	Product	Description
29068	ViaVac™ Red/Green, 10 mM in DMSO	Yeast vital dye
31062	Yeast Vitality Staining Kit	ViaVac™ Red/Green and Calcofluor White
31063	Yeast Viability Staining Kit	CF®-ConA and Live-or-Dye™ combinations
31064	Yeast Fixable Live/Dead Staining Kit	Thiazole Orange and Live-or-Dye™ 568/583

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