Biotium
Glowing Products for Science™

Apoptosis, Necrosis and Cell Viability Assays

MitoView™ Mitochondrial Dyes

Loss of mitochondrial membrane potential is a hallmark for apoptosis. It is an early event preceding phosphatidylserine externalization and coinciding with caspase activation.[1] Biotium offers novel and classic dyes for measuring mitochondrial membrane potential.

MitoView™ 633

MitoView™633 is a novel far-red fluorescent dye for the measurement of mitochondrial membrane potential (excitation/emission at 622/648 nm). Mitochondrial membrane potential and caspase-3 activity can be assayed together by fluorescence microscopy (Fig. 1-2) or flow cytometry (Fig. 3) using the NucView™ 488 and MitoView™ 633 Apoptosis Kit (see page 5).

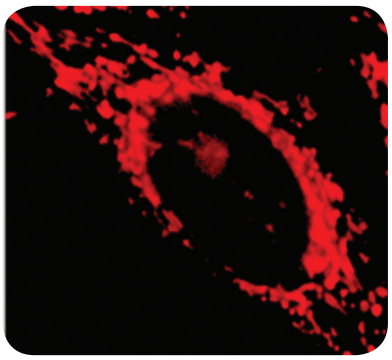

Figure 1. HeLa cell stained with MitoView™ 633.

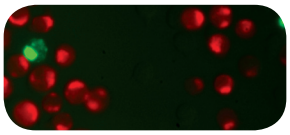

Figure 2. Jurkat cells stained with MitoView™633 and NucView™488 Apotosis Kit. Healthy cells stain red with MitoView 633, while apoptotic cells stain green with NucView 488 Caspase-3 Substrate. See pages 4-5 for more information on NucView™488 Caspase-3 Substrate.

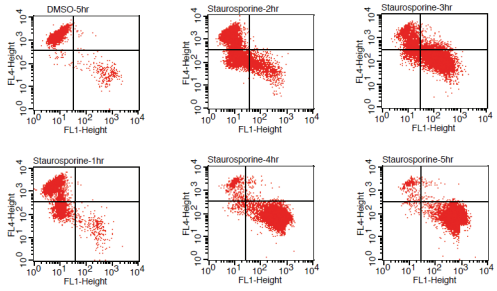

Figure 5. Flow cytometry analysis of staurosporine-treated Jurkat cells using NucView™488 and MitoView™633 Apoptosis Kit. Fluorescence was analyzed on a BD FACSCalibur flow cytometer. As apoptosis progresses, NucView™488 signal (FL1) increases while mitochondrial membrane potential measured by MitoView™633 staining (FL4) decreases. See pages 4-5 for more information on MitoView™633.

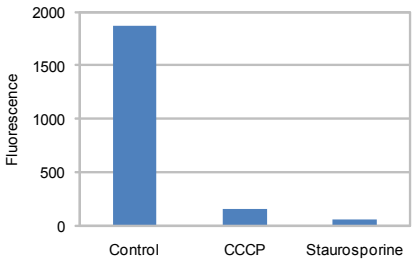

Figure 3. Flow cytometry of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in a significant decrease in MitoView™ 633 staining.

MitoView™ Green

MitoView™ Green is a non-potentiometric mitochondrial membrane dye. Cell staining with MitoView Green relies on mitochondrial mass, not membrane potential. Thus, the dye can be used to stain mitochondria in both live cells and fixed cells with green fluorescence (Fig. 4), and as a control to visualize mitochondria after depolarization.

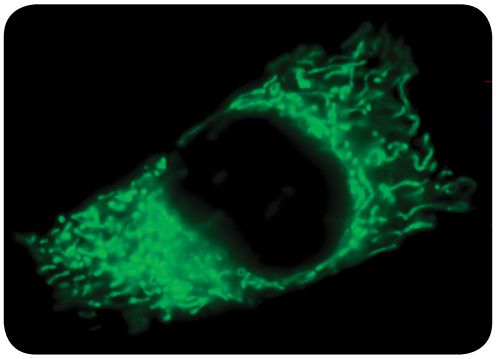

Figure 4. HeLa cell stained with MitoView™ Green.

JC-1 Mitochondrial Membrane Potential Detection Kit
In healthy cells, JC-1 dye aggregates in mitochondria as a function of membrane potential, resulting in red fluorescence (excitation/emission 585/590 nm) 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 (excitation/emission 510/527 nm)[2-5], allowing of cell viability to be assessed by measuring the ratio of red to green fluorescence by flow cytometry or fluorescence plate reader.

JC-1 dye also is available as a stand-alone product. JC-1 iodide salt is the original form of the dye; JC-1 chloride salt was developed for applications where iodide ion is not desired.

Rhodamine 123 is a green fluorescent mitochondrial dye (excitation/emission 505/534 nm) commonly used for flow cytometry measurement of mitochondrial membrane potential.[6-8]

TMRE and TMRM are cell permeable ethyl and methyl esters of tetramethylrhodamine, a red fluorescent dye (excitation/emission 548/573 nm) that accumulates in active mitochondria (Figure 4). These dyes are useful for flow cytometry measurement of mitochondrial membrane potential.[9,10]

DASPEI is a red fluorescent potentiometric mitochondrial dye (excitation/emission 461/589 nm) that has been used in no-wash assays for high content screening.[11]

DiIC$_1$(5) is a deep/far red carbocyanine dye (excitation/emission 638/658 nm), which has been used to measure mitochondrial membrane potential in apoptotic cells.[12]

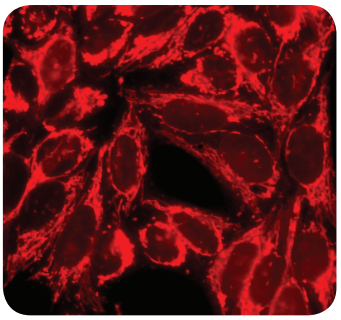

Figure 4. Live HeLa cells stained with the potentiometric mitochondrial dye TMRM.

MCB Glutathione Detection Kit
Diminished cellular glutathione (GSH) level occurs early in apoptosis due to GSH efflux from mitochondria.[13, 14] Monochlorobimane (MCB), which reacts with thiols to form a blue fluorescent product (Fig. 5) allowing fluorometric quantitation of GSH in cell lysates (Fig. 6).[15]

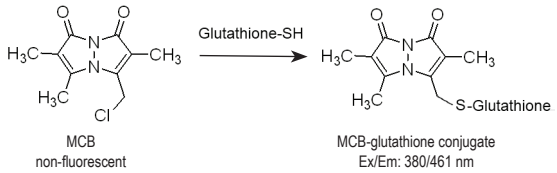

Figure 5. MCB glutathione assay principle.

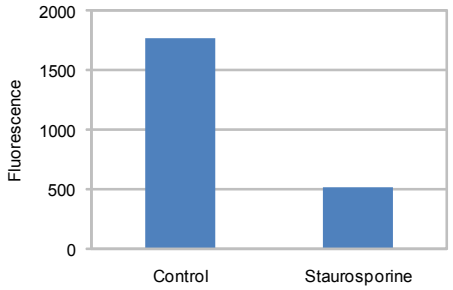

Figure 6. Jurkat cells were treated with DMSO (Control) or 1 uM staurosporine (Induced) for 5 hours. Glutathione levels were measured using the MCB Glutathione Detection Kit by fluorescence plate reader.

Ordering Information

Product description	Catalog number	Unit Size
NucView 488 and MitoView 633 Apoptosis Kit	30062	100 assays
Mitoview 633	70055	20 x 50 ug
MitoView™ Green	70054	20 x 50 ug
JC-1 Mitochondrial Membrane Detection Kit	30001	100 assays
JC-1, chloride salt	70011	5 mg
JC-1, iodide salt	70014	5 mg
MCB Glutathione Detection Kit	30019	100 assays
Rhodamine 123	70010	50 mg
Tetramethylrhodamine ethyl ester, perchlorate (TMRE)	70016	25 mg
Tetramethylrhodamine methyl ester, perchlorate (TMRM)	70017	25 mg
DASPEI	70018	100 mg
DiIC1(5)	70015	100 mg

References

1) Science 281, 1309-12 (1998); 2) Cytometry 29, 97 (1997); 3) FEBS Lett 411, 77 (1997); 4) J Neurochem 70, 66 (1998); 5) Biochemistry 30, 4480 (1991); 6) Cytometry 17, 50 (1994); 7) Science 218, 1117 (1982); 8) J Cell Biol 88, 526 (1981); 9) Cytometry 71A, 668 (2007); 10) Cytometry 45, 151 (2001); 11) J Biomol Screen 5, 1071 (2010); 12) Cytometry 33, 333 (1998); 13) Faseb J 12, 479 (1998); 14) Biochem Soc Trans 28, 56 (2000); 15) Cancer Res 46, 6105 (1986).

NucView™ 488 Caspase-3 Substrate for real-time detection of caspase-3 activity in intact cells

Proteolysis of cellular substrates by caspase-3 results in the morphological and biochemical features of apoptosis.[1] NucView™ 488 Caspase-3 Substrate is a novel cell membrane-permeable fluorogenic caspase substrate designed for detecting caspase-3 activity in real time.[2]

Traditional fluorogenic caspase substrates[3] require cell lysis and cannot be used to measure caspase activity in live cells; furthermore such assays measure only the average caspase activity in a cell population. Fluorescently-labeled caspase inhibitor assay (FLICA) reagents can enter live cells to detect caspase activity[4], but because the fluorescent probes are also irreversible caspase inhibitors, they cannot be used to follow caspase activity in real time.

NucView™ 488 Caspase-3 Substrate consists of a fluorogenic DNA dye and a DEVD substrate moiety specific for caspase-3. 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 DNA dye migrates to the cell nucleus to stain the nucleus with bright green fluorescence (Figs. 1,2). Detection of caspase-3 using NucView™488 has been reported in a wide variety of immortalized and primary cell types (Tables 1 and 2).

NucView™ 488 Caspase-3 Substrate is offered as a 1 mM stock solution in DMSO or PBS. DMSO facilitates NucView™ 488 Caspase-3 staining in some cell types. The PBS stock is offered for use in DMSO-sensitive cell types.

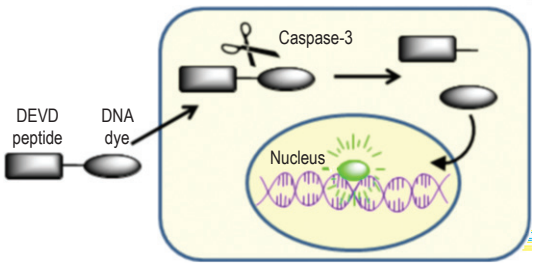

Figure 1. Principal of NucView™488 Caspase-3 Substrate staining

NucView™ enzyme substrate technology is covered under U.S. Patent No. 8,092,784. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to btinfo@biotium.com.

NucView™ Key Features:

- Bifunctional: allows caspase-3 detection and visualization of apoptotic nuclear morphology
- Does not interfere with caspase-3 activity, allowing real time caspase-3 monitoring[2]
- Rapid staining in cell culture medium with no washing required
- Formaldehyde-fixable, compatible with immunostaining[5]
- Detectable by fluorescence microscopy, flow cytometry, or fluorescence plate reader
- For use in adherent or suspension cells

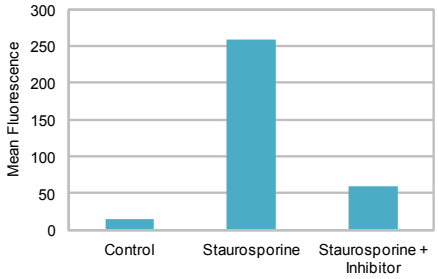

Figure 2. Flow cytometry analysis of caspase-3 activity in Jurkat cells using NucView™488 Caspase-3 Substrate. Cells were left untreated (control) or apoptosis was induced with 1 uM staurosporine for 4 hours (staurosporine). Cells were stained using the NucView™488 Caspase-3 Assay Kit, with or without caspase-3 inhibitor Ac-DEVD-CHO according the kit protocol. Fluorescence was measured in the FL1 channel of a BD FACSCalibur flow cytometer. Bars represent the mean fluorescence of the cell populations.

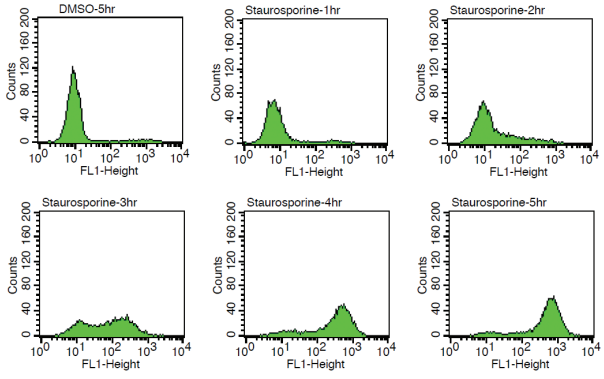

Figure 3. Flow cytometry analysis of caspase-3 activity in Jurkat cells using NucView™488. Jurkat cells were treated with 1 uM staurosporine for various times as indicated, or with DMSO as a control, then stained with NucView™488 and analyzed by flow cytometry in FL1 of a BD FACSCalibur cytometer. Histograms show the timecourse of NucView™488 staining during apoptosis induction with staurosporine.

*Table 1. Cell lines tested with NucView 488 caspase-3 substrate**

293-H	CCL-190	HUH6	MES-SA/DX	SKLMS1
293-T	GE11	Jurkat	Min 6	SW684
4T1	H9c2	JY	N19	SW872
67NR	HaCaT	K562	NRK	TK6
A172	HCLE	LLC-PK1	NRK-52E	U2OS
A204	HeLa	MCF-7	PC-3	U251
B16F10	HepT1	MDA-MB-231	PC12	U373 MG
BeWo	HMEC	MDCK	RD	WEHI 7.2
CCL-134	HT-1080	MES-SA	RINm5F	

**Based on published reports. Email techsupport@biotium.com to request a list of references.*

NucView™488 Caspase-3 Assay Kits

NucView™ 488 Caspase-3 Assay Kit for Live Cells contains substrate stock in DMSO and caspase-3 inhibitor Ac-DEVD-CHO.

Dual Apoptosis Assays with NucView™ 488 Caspase-3 Substrate and Annexin V include NucView caspase-3 substrate and either deep red fluorescent CF™594 Annexin V or far-red fluorescent CF™640R Annexin V for dual detection of caspase-3 activity and phosphatidylserine translocation in intact cells (Fig. 4). CF™594 and CF™640R are brighter and more photostable alternatives to Texas Red® and Cy®5, respectively.

NucView™ 488 and MitoView™ 633 Apoptosis Kit includes far-red fluorescent MitoView™ 633 mitochondrial membrane potential dye for simultaneous detection of caspase-3 activity and mitochondrial membrane potential (see p. 2).

NucView™488 and RedDot™2 Apoptosis & Necrosis Assay Kit pairs NucView™488 Caspase-3 substrate with the dead cell selective far-red dye RedDot™2 to stain necrotic and late apoptotic cells that have compromised membrane integrity (Fig. 5; see p. 10).

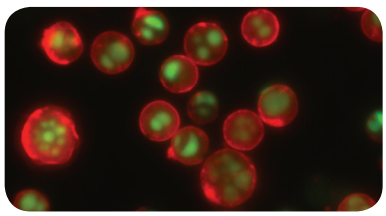

Figure 4. Staurosporine-treated apoptotic Jurkat cells stained using the Dual Apoptosis Assay with NucView™ 488 caspase-3 substrate (green) and CF™594 Annexin V (red).

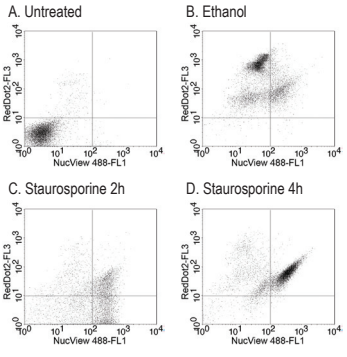

Figure 5. Flow cytometry analysis of Jurkat cells left untreated (A), treated with 10% ethanol for 90 minutes to induce necrosis (B), or treated with 1 uM staurosporine for 2 hours (C) or 4 hours (D) to induce apoptosis. NucView 488 was detected in the FL1 channel (488 nm excitation and 530/30 nm emission filter) and RedDot 2 in the FL3 channel (488 nm excitation/670 longpass emission filter) of a BD FACSCalibur flow cytometer. Necrotic cells stain low with NucView 488 and high with RedDot 2. Early apoptotic cells stain high with NucView 488 and low with RedDot 2, while late apoptotic cells stain high with both NucView 488 and RedDot 2.

*Table 2. Primary cells tested with NucView 488 caspase-3 substrate**

Alveolar epithelial cells (mouse)	Idiopathic pulmonary fibrosis fibroblasts (human)	SVZ neural progenitor cells (rat)	Retinal pigmented epithelial cells (human, mouse)
Dendritic cells (mouse)	Immature B-cells (mouse)	Oligodendrocytes (mouse)	Skin fibroblasts (sand cat)
Embryonic fibroblasts (mouse)	Kidney epithelial cells (mouse)	Oocytes (bovine, mouse)	Thymocytes (mouse)
Embryo tailbud (chicken)	Lung microvascular endothelial cells (human)	Pancreatic acinar cells (mouse)	Umbilical vein endothelial cells (human)
Hepatocytes (rat)	Macrophages (mouse)	Pancreatic beta cells (rat)	
Hemocytes (silkworm)	Mammary epithelial 3-D cultures (mouse)	Peritoneal macrophages (mouse)	
Hippocampal neurons (rat)	Neutrophils (human)	Pollen tubes (field poppy)	

**Based on published reports. Email techsupport@biotium.com to request a list of references.*

Additional caspase substrates and inhibitors

Biotium offers rhodamine 110 (R110)-based assay kits for green fluorescent or colorimetric detection of caspase-3 activity in cell lysates[3], and R110-based homogenous caspase-3 assay kits for high throughput screening. Biotium also offers a coumarin (AMC)-based blue fluorogenic substrate for measuring caspase activity in cell lysates[4].

Ac-DEVD-CHO is a competitive inhibitor of caspase-3 for use in cultured cells (Fig. 2) or cell lysates.[5]

Ordering Information

Product description	Catalog number	Unit 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
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
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	1 mL (10 assays)
	30009-2	10 mL (100 assays)
	30009-3	100 mL (1000 assays)
Ac-DEVD-AMC	10202	5 mg
Ac-DEVD-CHO Caspase-3 Inhibitor	10404-1	1 mg
	10404	5 mg

References
1) Cell Death Differ 6, 1067 (1999); 2) FASEB J 22, 243 (2008); 3) Biochemistry 38, 13906 (1999); 4) Biochemistry 39, 16056 (2000); 5) Int Immunol 8, 1173 (1996).

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 is available for annexin V binding.[1] Fluorescent conjugates of Annexin V can be used to detect apoptotic cells by fluorescence microscopy (Fig. 1) or flow cytometry (Fig. 2). Biotium offers a broad range of annexin V conjugates featuring our exceptionally bright and photostable CF™ dyes as well as assay kits for the differentiation of apoptotic and necrotic cells. CF™488A green fluorescent Annexin V conjugate is much brighter and more photostable than the traditional FITC-Annexin V, allowing the use of 10-fold less conjugate in staining. Near-infrared CF dye conjugates of Annexin V are supplied lyophilized and preservative-free, and are suitable for in vivo imaging.

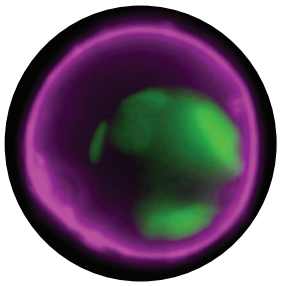

Figure 1. Apoptotic Jurkat cell stained with NucView™ 488 (green) and CF™647-Annexin V (magenta). See page 4 for information on NucView™ 488 Caspase-3 Substrate.

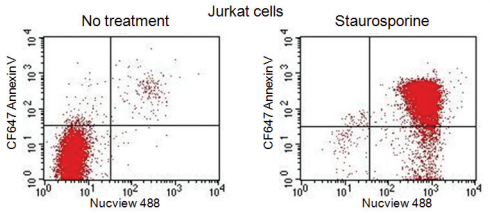

Figure 2. Flow cytometry analysis of untreated and staurosporine-treated Jurkat cell stained with NucView™ 488 and CF™647-Annexin V.

Dual apoptosis assay kit

Annexin V conjugated to our deep red CF™594-Annexin V is offered together with NucView™488 Caspase-3 Substrate[2] for simultaneous detection of caspase-3 activity and phosphatidylserine exposure by fluorescence microscopy or flow cytometry (see page 5 for more information).

Ordering Information

Product description	Ex/Em (nm)	Catalog number	Unit size
Annexin V, CF™350, 50 ug/mL	347/448	29012	0.5 mL
Annexin V, CF™405M, 50 ug/mL	408/452	29009	0.5 mL
Annexin V, CF™488A, 50 ug/mL	490/515	29005	0.5 mL
Annexin V, CF™555, 50 ug/mL	555/565	29004	0.5 mL
Annexin V, CF™568, 50 ug/mL	562/583	29010	0.5 mL
Annexin V, CF™594, 50 ug/mL	593/614	29011	0.5 mL
Annexin V, CF™633, 50 ug/mL	630/650	29008	0.5 mL
Annexin V, CF™640R, 50 ug/mL	642/662	29014	0.5 mL
Annexin V, CF™647, 50 ug/mL	650/665	29003	0.5 mL
Annexin V, CF™680, lyophlized	681/698	29007	25 ug
Annexin V, CF™750, lyophilized	755/777	29006	25 ug
Annexin V, CF™770, lyophilized	770/797	29046	25 ug
Annexin V, CF™790, lyophilized	784/806	29047	25 ug
Annexin V, FITC, 50 ug/mL	490/525	29001	0.5 mL
Anexin V, R-phycoerythrin (R-PE)	496, 546, 565/578	29045-100 uL	100 uL (20 assays)
		29045-500 uL	500 uL (100 assays)
Annexin V, Texas Red®, 50 ug/mL	596/615	29002	0.5 mL
Annexin V, biotin, 50 ug/mL	N/A	29013	0.5 mL
5X Annexin V Binding Buffer	N/A	99902	15 mL

References
1) J Nuc Med 49, 1573 (2008); 2) FASEB J 22, 243 (2008).

Apoptosis & Necrosis Quantitation Kits

Biotium's apoptosis/necrosis quantitation kits pair green fluorescent CF™488A-Annexin V with a selection of membrane impermeant red fluorescent nucleic acid dyes to distinguish early apoptotic cells from late apoptotic and necrotic cells with compromised membrane integrity. The nucleic acid dyes also allow visualization of nuclear morphology to distinguish late apoptotic cells with compromised plasma membranes from necrotic cells (Fig. 1). CF™488 is significantly brighter and more photostable than traditional green dyes like fluorescein.

Apoptosis Kits with CF™488A-Annexin V and 7-AAD or Propidium Iodide
These kits pair green fluorescent CF™488 Annexin V for detection of apoptotic cells with a red fluorescent vital dye for detection of necrotic and late apoptotic cells with compromised membrane integrity. 7-AAD is useful for fluorescence microscopy (Fig. 1), due to minimal fluorescence spill-over of 7-AAD in the green channel, while propidium iodide is recommended for flow cytometry (Fig. 2).

Apoptosis & Necrosis Quantitation Kit Plus and Apoptotic, Necrotic, and Healthy Cells Quantitation Kit Plus
These kits feature ethidium homodimer III,[1,2] 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 Hoechst 33342, a membrane permeable blue fluorescent DNA dye (Ex/Em with DNA 350/461 nm) to allow visualization of the total cell population (Fig. 3).[3,4]

Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells

This kit includes calcein-AM, which stains viable cells with green fluorescence,[5,6] and ethidium homodimer III[1,2] which stains dead cells. For detection of live and dead cells by microscopy, flow cytometry, or fluorescence microplate reader. See page 9 for more information on calcein-AM.

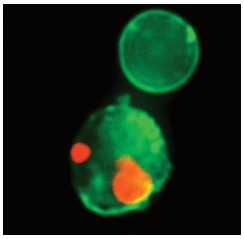

Figure 1. Staurosporine-treated Jurkat cells stained with CF™488A Annexin V (green) and 7-AAD (red).

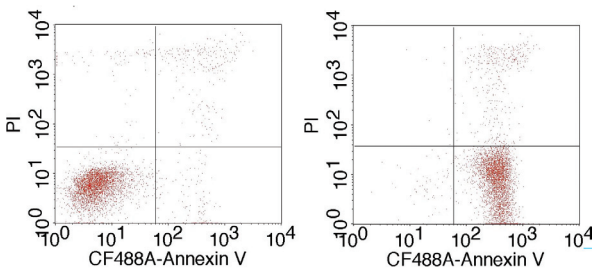

Figure 2. Flow cytometry analysis of untreated and staurosporine-treated Jurkat cell stained with CF™488A Annexin V (FL1) and PI (FL3).

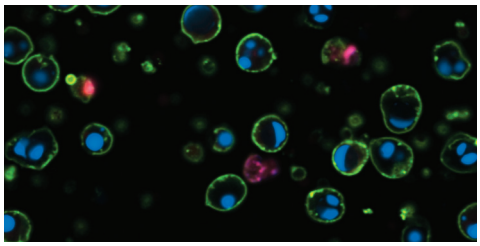

Figure 3. Jurkat cells stained using the Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus after apoptosis induction with 1 uM staurosporine for 4 hours.

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.[7]

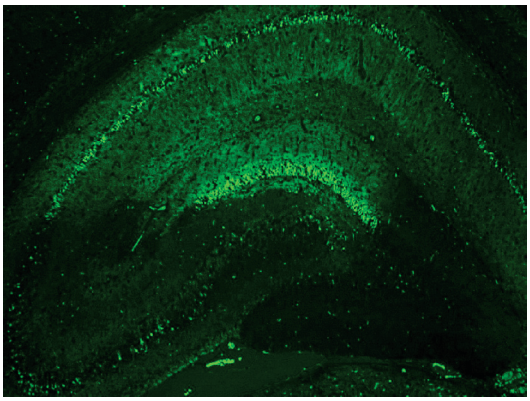

Figure 4. Degenerating neurons in a section of mouse hippocampus stained with PathoGreen™ Histofluorescent Stain.

Ordering Information

Product description	Catalog number	Unit Size
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
Viability and Cytotoxicity Kit for Animal Live & Dead Cells	30002-T	150 assays
	30002	300 assays
PathoGreen™ Histofluorescent Stain, 1000X in water	80027-5mL	5 mL
	80027-50 mL	50 mL

References
1) Mol Cancer Res 6, 965 (2008); 2) J Pharmacol & Exper Therap 332, 738 (2010); 3) Science 300, 91 (2003); 4) J Neurosci Methods 36, 229 (1991); 5) J Immunol Methods 177, 101 (1994); 6) Hum Immunol 37, 264 (1993) 7) Toxicol Pathol 28, 91 (2000).

Internucleosomal cleavage of DNA is a hallmark of apoptosis[1]. DNA cleavage in apoptotic cells can be detected in situ in fixed cells or tissue sections by TUNEL labeling, which 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.[2]

In the terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick-end labeling (TUNEL) 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.

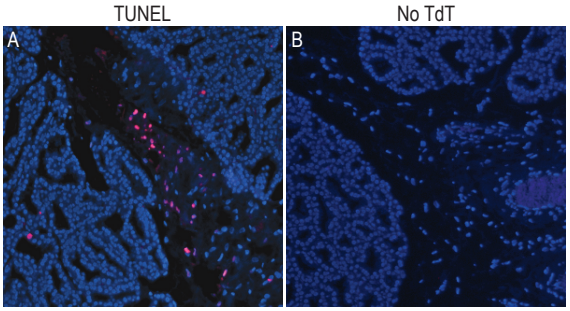

Figure 2. A. TUNEL staining of paraffin sections of rat mammary gland 5 days post-weaning (ApopTag® positive control slides, Millipore) using far-red CF640R-dUTP (red). B. Negative control TUNEL reaction with TdT enzyme omitted. Nuclei are counterstained with DAPI (blue).

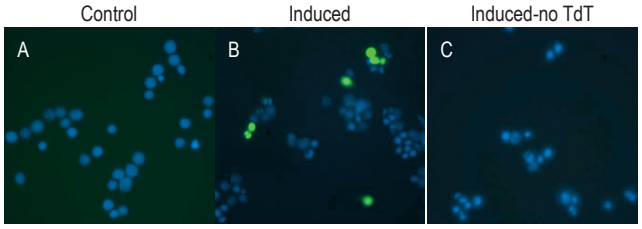

Figure 1. Jurkat cells labeled using the CF™488 TUNEL Assay Apoptosis Detection Kit after no treatment (A) or apoptosis induction with 1 uM staurosporine for 3 hours (B). Specificity of TUNEL labeling (green) is demonstrated by omission of TdT enzyme (C). Nuclei are counterstained with DAPI (blue).

Biotin-dUTP conjugates

Biotium offers a number of biotin-dUTP with different linker lengths between the biotin and dUTP. In general, shorter linker dUTP conjugates are incorporated into DNA more efficiently, while longer linker conjugates interact better with CF™ dye-labeled streptavidin. The numbering of the conjugates refers to the length of the linker; for example, biotin-11-dUTP has an eleven atom linker.

CF™ Dye dUTP conjugates and TUNEL Kits

Biotium offers dUTP conjugated to a range of CF™dye colors for direct fluorescent TUNEL labeling. Our CF™488A and CF™594 TUNEL Assay Apoptosis Detection Kits contain complete reaction buffer and TdT enzyme for TUNEL labeling using our exceptionally bright and photostable green fluorescent CF™488A or deep red fluorescent CF™594, for bright fluorescent TUNEL staining using a convenient, rapid, direct labeling protocol.

Ordering Information

Product description	Ex/Em (nm)	Catalog number	Unit Size
CF™488A TUNEL Assay Apoptosis Detection Kit	490/515	30063	50 reactions
CF™594 TUNEL Assay Apoptosis Detection Kit	593/614	30064	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
Biotin-11-dUTP, 1 mM in pH 7.5 Tris-HCl buffer	N/A	40029	50 uL
Biotin-11-dUTP, lyophilized powder	N/A	40029-1	50 ug
Biotin-16-dUTP, 1 mM in pH 7.5 Tris-HCl buffer	N/A	40022	50 uL
Biotin-16-dUTP, lyophilized powder	N/A	40022-1	50 ug
Biotin-20-dUTP, 1 mM in pH 7.5 Tris-HCl buffer	N/A	40030	50 uL
Biotin-20-dUTP, lyophilized powder	N/A	40030-1	50 ug

References
1) Chromosoma 115: 89-97 (2006); 2) Lab Invest 71(2):219-25 (1994).

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.[1,2] 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.[3,4]

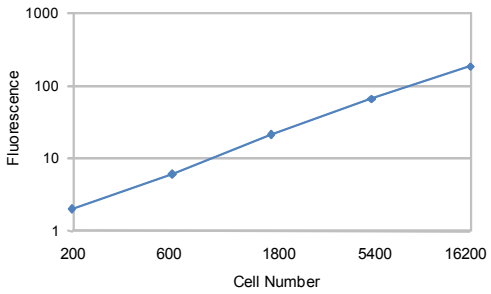

Figure 1. Quantitation of HeLa cell numbers using the Calcein AM Cell Viability Assay Kit. Cells were plated in 96-wells 24 hours before assay.

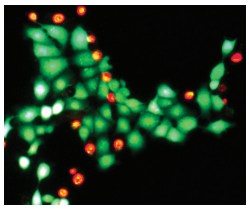

Figure 2. Live and dead HeLa cells stained with the Viability/Cytotoxicity Assay for Animal Live & Dead Cells. Live cells are stained green, dead cells are stained red.

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.[8-10] 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 assay is 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.

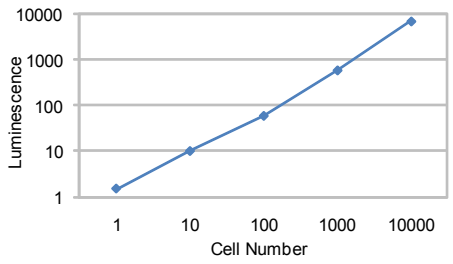

Figure 3. Quantitation of 10-fold serial dilutions of Jurkat cells in suspension using ATP-Glo™ Bioluminetric Cell Viability Assay using a single-sample luminometer.

Resazurin, MTT, and XTT Viability Assays

MTT, XTT, and resazurin (Alamar Blue®) are reduced by mitochondrial metabolic activity to yield colored or fluorescent products, and thus are useful for and assaying cell viability and quantitating cell number. MTT and XTT are reduced to colored formazin salts that can be measured by absorbance [5,6]. 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.[7]

Cell Proliferation Dyes

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.

CFDA SE Cell Proliferation Dye is hydrolyzed in cells to release green fluorescent carboxy-fluorescein, for detection in the FITC channel. CFDA SE is available in a kit with 10 x 50 ug dye, anhydrous DMSO, and a detailed protocol, or as a stand-alone dye.

Ordering Information

Product description	Catalog number	Unit Size
Calcein AM Cell Viability Assay Kit	30026	1000 assays
Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells	30002-T	150 assays
	30002	300 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
ViaFluor™405-SE Cell Proliferation Kit	30068	1 kit
CFDA SE Cell Proliferation Assay Kit	30050	1 kit
(5,6) CFDA, SE	90041	25 mg

References
1) J Immunol Methods 177, 101 (1994); 2) Hum Immunol 37, 264 (1993); 5) J Immunol Methods 65, 55 (1983); 6) J Immunol Meth 159, 81 (1993); 7) J Immunol Meth 170, 211 (1994); 8) J Immunol Meth 160, 81 (1993); 9) J Biolumin Chemilumin 10, 29 (1995);
10) Toxicology In Vitro 11, 553 (1997).

Vital Dyes, Apoptosis Inducers, Bacterial Viability

Vital dyes
Ethidium homodimer III[1,2] 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, while RedDot™2 selectively stains cells with compromised membrane integrity. RedDot™2 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.

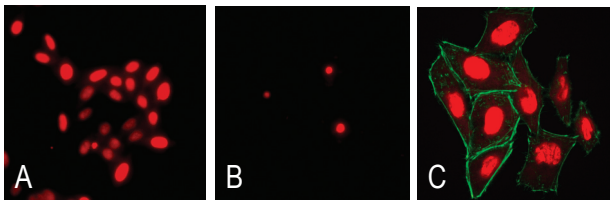

Figure 1. A. Nuclear staining of live HeLa cells with RedDot™1. B. Selective staining of dead HeLa cells with RedDot™2. C. Fixed and permeabilized HeLa cells stained with RedDot™2. Actin filaments are stained green with CF™488A phalloidin.

Ordering Information

Product description	Catalog number	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
Ethidium Homodimer III	40050	1 mg
Ethidium Homodimer III, 1 mM in DMSO	40051	200 uL
Staurosporine	00025	100 ug
Ionomycin, calcium salt	59007	1 mg
Viability/Cytotoxicity Assay kit for Bacteria	30027	100-1000 assays
Bacterial Viability and Gram Stain Kit	32001	200 assays
PMA™ dye	40013	1 mg
PMA™ dye, 20 mM in water	40019	100 uL
PMA-Lite™ LED Photolysis Device	E90002	Each

Chemical inducers of apoptosis
Staurosporine is a broad range protein kinase inhibitor that induces apoptosis in cultured cells.[3-6] We also offer ionomycin, a calcium ionophore that has been shown to induce apoptosis through calpain activation.[4]

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.

PMA™ for selective detection of live cells
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. Thus, subsequent analysis by qPCR permits selective detection of live cell DNA (Figure 2).[5]

PMA-Lite™ LED Photolysis Device is specifically designed for photoactivation of PMA and other similar dyes. Receive a free vial of 20mM PMA in H_2O when you purchase a PMA-Lite™ LED Photolysis Device.

Features:
- 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
- For efficient activation of PMA™, EMA or other similar azido dyes.

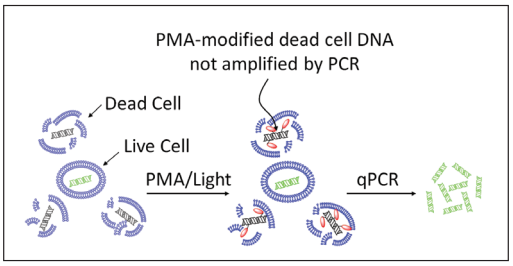

Figure 2. Principle of selective qPCR of live bacteria after treatment with PMA™ and light.

Figure 3. PMA-Lite™ LED Photolysis Device.

References
1) Mol Cancer Res 6, 965 (2008); 2) J Pharmacol Exp Ther 332, 738 (2010); 3) Biochem Biophys Res Commun 158, 105 (1989); 4) J Biol Chem 277, 27217 (2002); 5) J Microbiol Meth 67, 310 (2006).

Exceptionally bright and photostable fluorescent CF™dye conjugates

- Secondary antibodies and bioconjugates for immunofluorescence staining
- Near-infrared dye conjugates for Western blotting and in vivo imaging
- Reactive dyes and protein labeling kits
- Mix-n-Stain™ Antibody Labeling Kits for rapid antibody labeling
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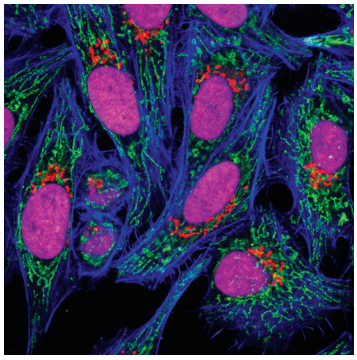

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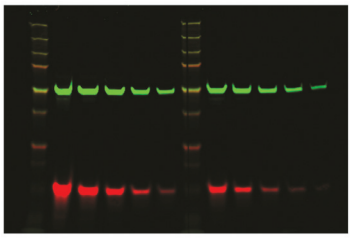
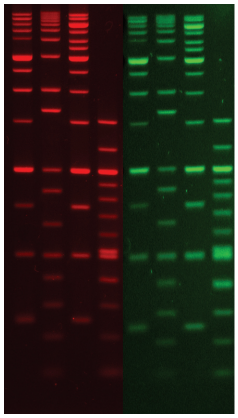
- Environmentally safe EvaGreen® qPCR dye and master mixes
- AccuBlue™ DNA quantitation kits
- AccuLite™ Mini-Fluorometers
- Lumitein™ fluorescent protein gel stain



Near-Infrared CF™dye conjugates

Other cell biology research tools:

- EverBrite™ antifade mounting medium with or without DAPI
- CoverGrip™ Coverslip Sealant: nail polish alternative
- Lipophilic dye membrane labeling kits
- Cytosolic and neuronal tracers
- Firefly and Renilla Luciferase Assay kits
- Aquaphile™ water soluble coelenterazines
- Enzyme substrates
- Fluorescent reagents for imaging calcium, pH, and other ions
- Biochemical reagents for detection of reactive oxygen species and nitric oxide



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