

## Apoptosis, Necrosis and Cell Viability Assays

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# Mitochondrial Membrane Potential Dyes

Loss of mitochondrial membrane potential is a hallmark for apoptosis. It is an early event preceding phosphatidylserine externalization and coinciding with caspase activation.<sup>1</sup> 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) or flow cytometry (Fig. 2) using the NucView™ 488 and MitoView™ 633 Apoptosis Kit (see page 5).

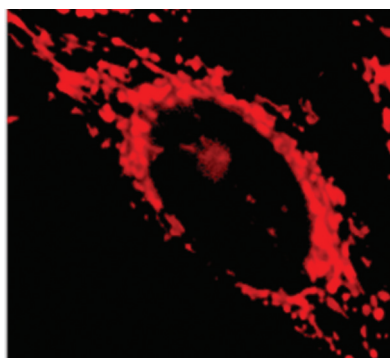


Figure 1. HeLa cell stained with MitoView™ 633.

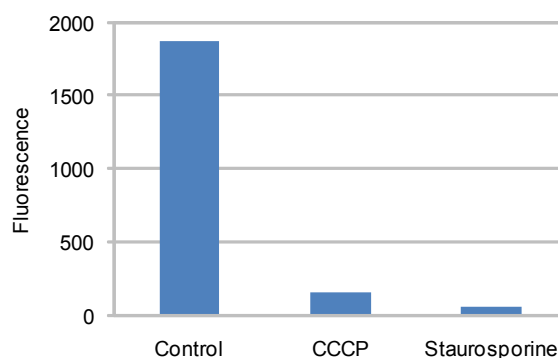


Figure 2. Flow cytometry of Jurkat cells treated with CCCP to depolarize the mitochondrial membrane or staurosporine to induce apoptosis, resulting in a significant decrease in MitoView™ 633 staining.

MitoView™ Green 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. 3), and as a control to visualize mitochondria after depolarization.

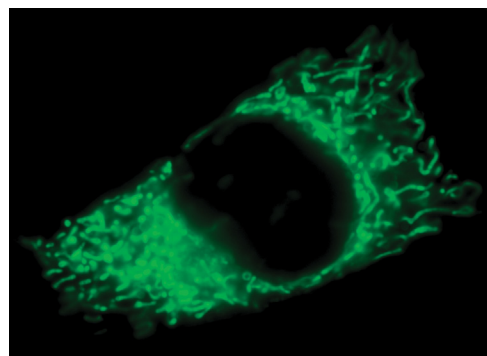


Figure 3. HeLa cell stained with MitoView™ Green.

## 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)<sup>2-5</sup>, allowing of cell viability to be assessed by measuring the ratio of red to green fluorescence by flow cytometry or fluorescence plate reader. Rhodamine 123 is a green fluorescent mitochondrial dye (excitation/emission 505/534 nm) commonly used for flow cytometry measurement of mitochondrial membrane potential.<sup>6-8</sup>

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. These dyes are useful for flow cytometry measurement of mitochondrial membrane potential.<sup>9,10</sup>

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.<sup>11</sup>

DiIC<sub>1</sub>(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.<sup>12</sup>

## MCB Glutathione Detection Kit

Diminished cellular glutathione (GSH) level occurs early in apoptosis due to GSH efflux from mitochondria.<sup>13, 14</sup> Monochlorobimane (MCB), which reacts with thiols to form a blue fluorescent product (Fig. 4) allowing fluorometric quantitation of GSH in cell lysates (Fig. 5).<sup>15</sup>

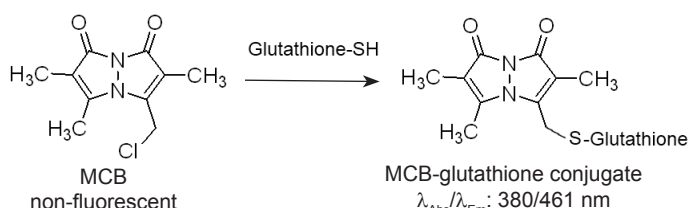


Figure 4. MCB glutathione assay principle.

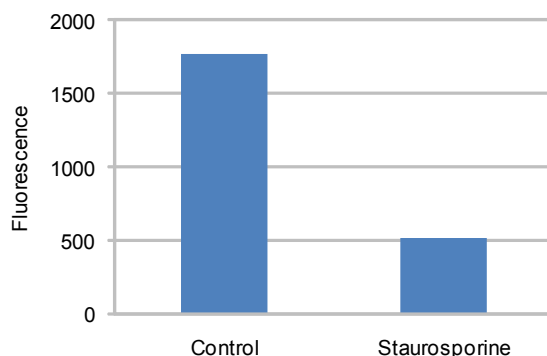


Figure 5. Jurkat cells were treated with DMSO (Control) or 1 μM staurosporine (Induced) for 5 hours. Glutathione levels were measured using the MCB Glutathione Detection Kit by fluorescence plate reader.

Catalog number	Product description
30062	NucView 488 and MitoView 633 Apoptosis Kit
70055	Mitoview 633
30001	JC-1 Mitochondrial Membrane Detection Kit
30019	MCB Glutathione Detection Kit
70010	Rhodamine 123
70016	Tetramethylrhodamine ethyl ester, perchlorate (TMRE)
70017	Tetramethylrhodamine methyl ester, perchlorate (TMRM)
70018	DASPEI
70015	DiIC <sub>1</sub> (5)
70054	MitoView™ Green

## 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).

# Caspase Assays

## 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.<sup>1</sup> NucView™ 488 Caspase-3 Substrate is a novel cell membrane-permeable fluorogenic caspase substrate designed for detecting caspase-3 activity in real time.<sup>2</sup>

Traditional fluorogenic caspase substrates<sup>3</sup> 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<sup>4</sup>, 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.

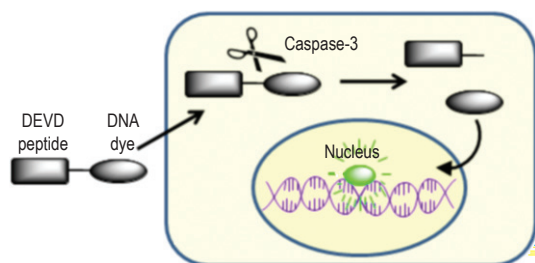


Figure 1. Principal of NucView™ 488 Caspase-3 Substrate staining

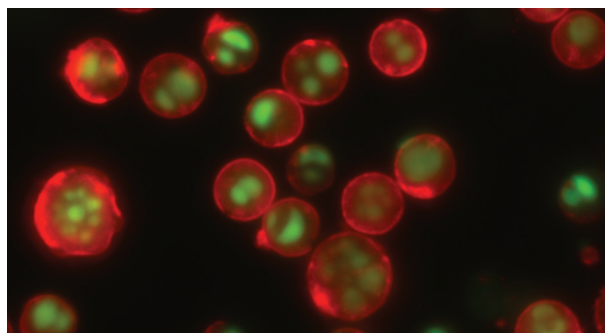


Figure 2. Staurosporine-treated apoptotic Jurkat cells stained using the Dual Apoptosis Assay with NucView™ 488 caspase-3 substrate (green) and CF™ 594 Annexin V (red).

### 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<sup>2</sup>
- Rapid staining in cell culture medium with no washing required
- Formaldehyde-fixable, compatible with immunostaining<sup>5</sup>
- Detectable by fluorescence microscopy, flow cytometry, or fluorescence plate reader
- For use in adherent or suspension cells



Table 1. Cell lines tested with NucView 488 caspase-3 substrate<sup>5</sup>

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

Table 2. Primary cells tested with NucView 488 caspase-3 substrate<sup>5</sup>

Mouse dendritic cells	Mouse kidney epithelial cells	Mouse pancreatic acinar cells	Sand cat skin fibroblasts
Mouse embryonic fibroblasts	Human lung microvascular endothelial cells	Rat pancreatic beta cells	Mouse thymocytes
Rat hepatocytes	Mouse macrophages	Mouse pancreatic islet cells	Human umbilical vein endothelial cells
Rat hippocampal neurons	Mouse mammary epithelial 3-D cultures	Mouse peritoneal macrophages	
Human idiopathic pulmonary fibrosis fibroblasts	Rat neural progenitor cells	Field poppy pollen tubes	
Mouse immature B-cells	Mouse oligodendrocytes	Human, mouse retinal pigmented epithelial cells	

## 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.

NucView™ 488 Caspase-3 Substrate and CF™ 594-Annexin V Dual Apoptosis Assay Kit includes deep red fluorescent CF™ 594-annexin V for dual detection of caspase-3 activity and phosphatidylserine translocation in intact cells (Fig. 3).

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 (Fig. 3).

## Additional caspase substrates

Biotium offers a coumarin (AMC)-based blue fluorogenic substrate for measuring caspase activity in cell lysates<sup>3</sup>.

## Caspase-3 inhibitor

Ac-DEVD-CHO is a competitive inhibitor of caspase-3 for use in cultured cells or cell lysates.<sup>4</sup>

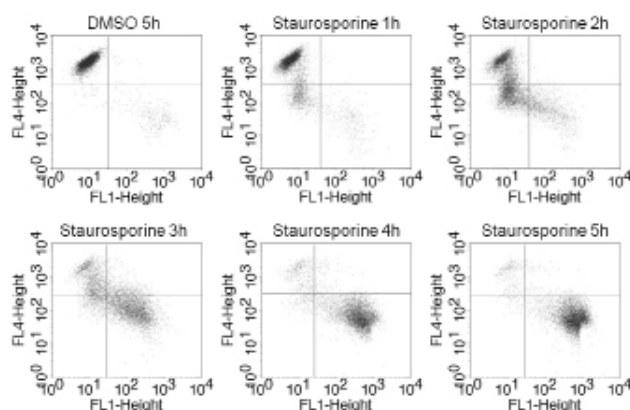


Figure 3. Flow cytometry analysis of staurosporine-treated Jurkat cells using NucView™ 488 and MitoView™ 633 Apoptosis Kit. Fluorescence was analyzed on a BD FACSCalibur flow cytometer. As apoptosis progresses, NucView™ 488 signal (FL1) increases while mitochondrial membrane potential measured by MitoView™ 633 staining (FL4) decreases.

Catalog number	Product description
30029	NucView™ 488 Caspase-3 Assay Kit for live cells
30067	Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™ 594-Annexin V
30062	NucView™ 488 and MitoView™ 633 Apoptosis Kit
10402	NucView™ 488 Caspase-3 Enzyme Substrate 1 mM in DMSO
10403	NucView™ 488 Caspase-3 Enzyme Substrate 1 mM in PBS
10404	Ac-DEVD-CHO Caspase-3 Inhibitor, 5 mg
10404-1	Ac-DEVD-CHO Caspase-3 Inhibitor, 1 mg
10202	Ac-DEVD-AMC, 5 mg

## References

1) Cell Death Differ 6, 1067 (1999); 2) FASEB J 22, 243 (2008); 3) Biochemistry 39, 16056 (2000); 4) Int Immunol 8, 1173 (1996); 5) Email techsupport@biotium.com to request a list of references.

# 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.<sup>1</sup> 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.

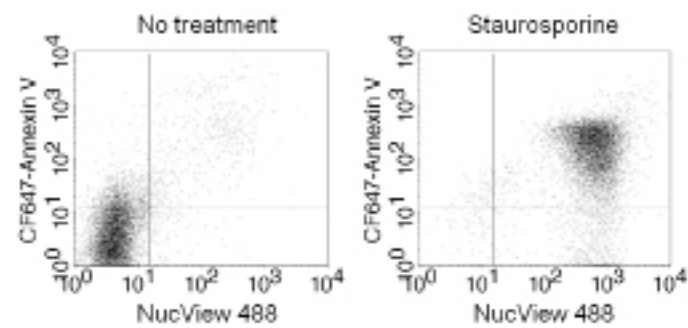


Figure 2. Flow cytometry analysis of untreated and staurosporine-treated Jurkat cell stained with NucView™ 488 and CF™647-Annexin V.

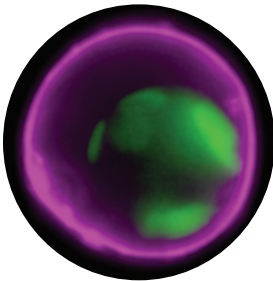


Figure 1. Apoptotic Jurkat cell stained with NucView™ 488 (green) and CF™647-Annexin V (magenta). See page 4 for information on NucView™ 488 Caspase-3 Substrate.

## Dual apoptosis assay kit

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

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

Catalog number	Product description	Ex/Em (nm)
29012	Annexin V, CF™350 conjugate	347/448
29009	Annexin V, CF™405M conjugate	408/452
29005	Annexin V, CF™488A conjugate	490/515
29004	Annexin V, CF™555 conjugate	555/565
29010	Annexin V, CF™568 conjugate	562/583
29011	Annexin V, CF™594 conjugate	593/614
29008	Annexin V, CF™633 conjugate	630/650
29014	Annexin V, CF™640R conjugate	642/662
29003	Annexin V, CF™647 conjugate	650/665
29007	Annexin V, CF™680 conjugate	681/698
29006	Annexin V, CF™750 conjugate	755/777
29001	Annexin V, FITC conjugate	490/525
29002	Annexin V, Sulforhodamine 101 (Texas Red®) conjugate	596/615
29013	Annexin V, biotin conjugate	N/A
99902	5X Annexin V Binding Buffer	N/A

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.

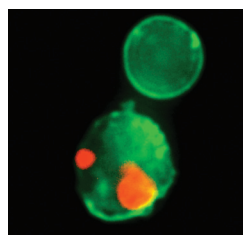


Figure 1. Staurosporine-treated Jurkat cells stained with CF™488A Annexin V (green) and 7-AAD (red).

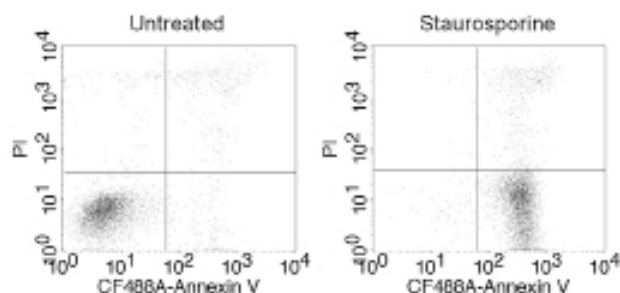


Figure 2. Flow cytometry analysis of untreated and staurosporine-treated Jurkat cell stained with CF™488A Annexin V (FL1) and PI (FL3).

## 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,<sup>1,2</sup> 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 Plus 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).<sup>3,4</sup>

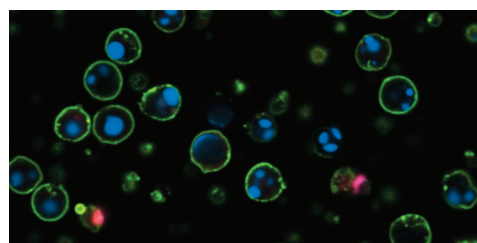


Figure 3. Jurkat cells stained using the Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus after apoptosis induction with 1 μM staurosporine for 4 hours.

Catalog number	Product description
30067	NucView™ 488 Caspase-3 Substrate and CF™594-Annexin V Dual Apoptosis Assay Kit
30065	Apoptosis & Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus
30060	CF™488A Annexin V and 7-AAD Apoptosis Kit
30061	CF™488A Annexin V and PI Apoptosis Kit

### 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).

# dUTP Conjugates and TUNEL Kits

Internucleosomal cleavage of DNA is a hallmark of apoptosis<sup>1</sup>. 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.<sup>2</sup>

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.

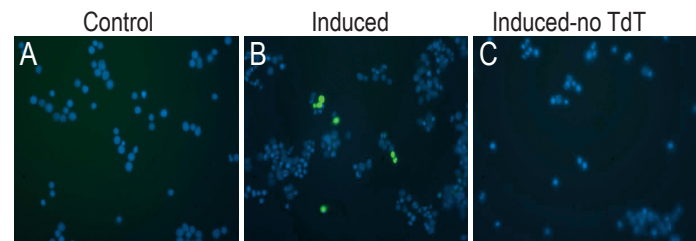


Figure 1. Jurkat cells labeled using the CF™488 TUNEL Assay Apoptosis Detection Kit after no treatment (A) or apoptosis induction with 1  $\mu$ M staurosporine for 3 hours (B). Specificity of TUNEL labeling (green) is demonstrated by omission of TdT enzyme (C). Nuclei are counterstained with DAPI (blue).

## 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.

### References

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

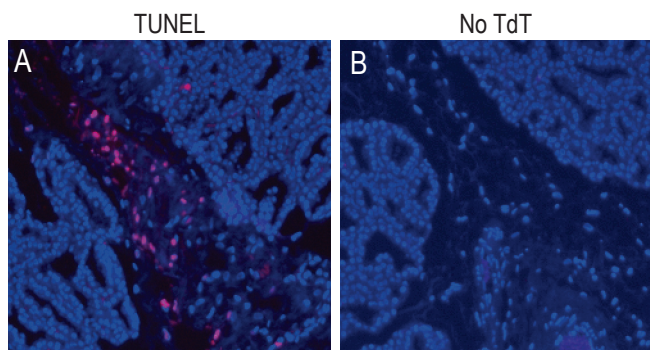


Figure 2. A. TUNEL staining of paraffin sections of rat mammary gland 5 days post-weaning (ApopTag® positive control slides, Millipore) using far-red CF640R-dUTP (red). B. Negative control TUNEL reaction with TdT enzyme omitted. Nuclei are counterstained with DAPI (blue).

## 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.

Catalog number	Product description
30063	CF™488A TUNEL Assay Apoptosis Detection Kit
30064	CF™594 TUNEL Assay Apoptosis Detection Kit
40004	CF™405S-dUTP
40008	CF™488A-dUTP
40002	CF™543-dUTP
40005	CF™568-dUTP
40006	CF™594-dUTP
40007	CF™640R-dUTP
40003	CF™680R-dUTP
40029	Biotin-11-dUTP, 1 mM in pH 7.5 Tris-HCl buffer
40029-1	Biotin-11-dUTP, lyophilized powder
40022	Biotin-16-dUTP, 1 mM in pH 7.5 Tris-HCl buffer
40022-1	Biotin-16-dUTP, lyophilized powder
40030	Biotin-20-dUTP, 1 mM in pH 7.5 Tris-HCl buffer
40030-1	Biotin-20-dUTP, lyophilized powder



## 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.<sup>1,2</sup> 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.<sup>3,4</sup>

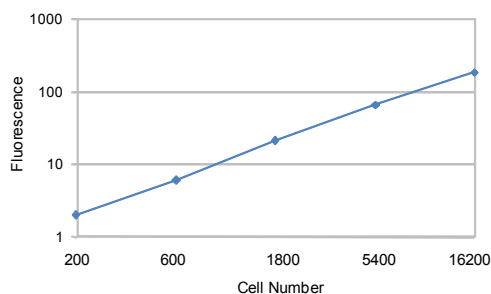


Figure 1. Quantitation of HeLa cell numbers using the Calcein AM Cell Viability Assay Kit. Cells were plated in 96-wells 24 hours before assay.

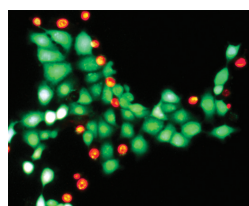


Figure 2. Live and dead HeLa cells stained with the Viability/Cytotoxicity Assay for Animal Live & Dead Cells. Live cells are stained green, dead cells are stained red.

## ATP-Glo™ Bioluminescent 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.<sup>8-10</sup> 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.

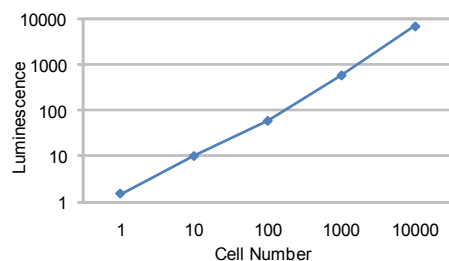


Figure 3. Quantitation of 10-fold serial dilutions of Jurkat cells in suspension using ATP-Glo™ Bioluminescent Cell Viability Assay using a single-sample luminometer.

## 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 formazan salts that can be measured by absorbance<sup>5,6</sup>. MTT generates an insoluble formazan 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.<sup>7</sup>

## 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.

Catalog number	Product description
30026	Calcein AM Cell Viability Assay Kit
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells
30025	Resazurin Cell Viability Assay Kit
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30020	ATP-Glo™ Bioluminescent Cell Viability Assay Kit
30068	ViaFluor™405-SE Cell Proliferation Kit
30050	CFDA SE Cell Proliferation Assay Kit
90041	(5,6) CFDA, SE, 25 mg

## References

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

Vital dyes

Ethidium homodimer III<sup>1,2</sup> 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](http://www.biotium.com) for more information.

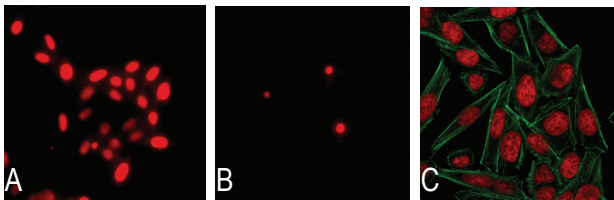


Figure 1. A. Nuclear staining of live HeLa cells with RedDot™1. B. Selective staining of dead HeLa cells with RedDot™2. C. Fixed and permeabilized HeLa cells stained with RedDot™2. Actin filaments are stained green with CF™ 488A phalloidin.

Catalog number	Product description
40060	RedDot™1
40061	RedDot™2
40051	Ethidium Homodimer III, 1 mM in DMSO
00025	Staurosporine
59007	Ionomycin, calcium salt
30027	Viability/Cytotoxicity Assay kit for Bacteria Live & Dead Cells
32001	Bacterial Viability and Gram Stain Kit
40019	PMA™ dye, 20 mM in water

Chemical inducers of apoptosis

Staurosporine is a broad range protein kinase inhibitor that induces apoptosis in cultured cells.<sup>3-6</sup> We also offer ionomycin, a calcium ionophore that has been shown to induce apoptosis through calpain activation.<sup>4</sup>

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).<sup>5</sup>

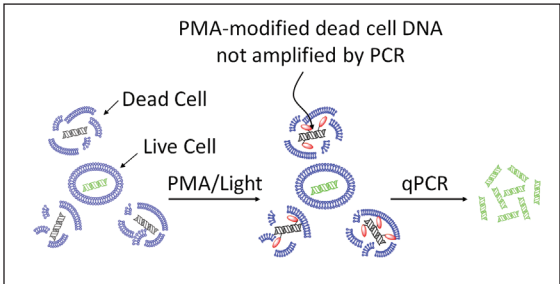
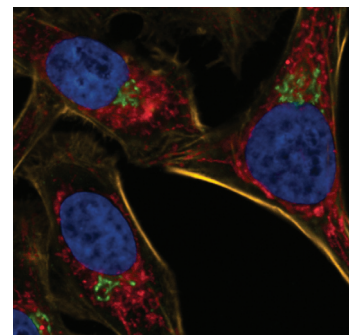


Figure 2. Principle of selective qPCR of live bacteria after treatment with PMA™ and light.

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

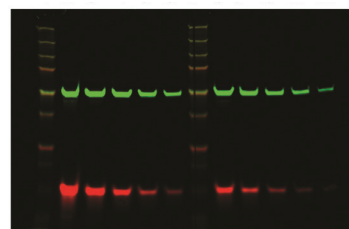
- Secondary antibodies and bioconjugates for immunofluorescence staining
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- Reactive dyes and protein labeling kits
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*CF™ dye-labeled secondary antibodies and bioconjugates*

### Genomics and proteomics products:

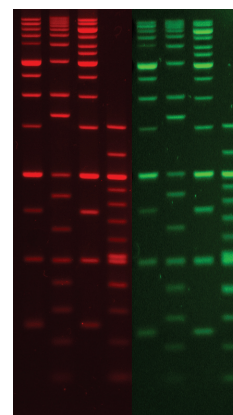
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- AccuBlue™ DNA quantitation kits
- Lumitein™ fluorescent protein gel stain



*Near-Infrared CF™ dye conjugates*

### Other cell biology research tools:

- EverBrite™ antifade mounting medium with or without DAPI
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*GelRed™ and GelGreen™ nucleic acid gel stains*

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