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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. 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.
Mitochondrial Membrane Potential Dyes

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. 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. 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.\(^12\)

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. 4) allowing fluorometric quantitation of GSH in cell lysates (Fig. 5).\(^15\)

**Figure 4.** MCB glutathione assay principle.

**Figure 5.** 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.

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<tr>
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<td>70055</td>
<td>Mitoview 633</td>
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<td>30001</td>
<td>JC-1 Mitochondrial Membrane Detection Kit</td>
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<td>30019</td>
<td>MCB Glutathione Detection Kit</td>
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<td>70010</td>
<td>Rhodamine 123</td>
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<td>70016</td>
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<td>70018</td>
<td>DASPEI</td>
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<td>70015</td>
<td>DiIC(_1)(_5)</td>
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<tr>
<td>70054</td>
<td>MitoView™ Green</td>
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References
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. NucView™ 488 Caspase-3 Substrate is a novel cell membrane-permeable fluorogenic caspase substrate designed for detecting caspase-3 activity in real time.

Traditional fluorogenic caspase substrates 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, 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 moity 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.

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

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).
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 lysates3.

Caspase-3 inhibitor
Ac-DEVD-CHO is a competitive inhibitor of caspase-3 for use in cultured cells or cell lysates4.

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.

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

<table>
<thead>
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<th>293-H</th>
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Table 2. Primary cells tested with NucView 488 caspase-3 substrate

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<th>Mouse dendritic cells</th>
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<th>Mouse pancreatic acinar cells</th>
<th>Sand cat skin fibroblasts</th>
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<td>Human lung microvascular endothelial cells</td>
<td>Rat pancreatic beta cells</td>
<td>Mouse thymocytes</td>
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<td>Rat hepatocytes</td>
<td>Mouse macrophages</td>
<td>Mouse pancreatic islet cells</td>
<td>Human umbilical vein endothelial cells</td>
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<td>Rat hippocampal neurons</td>
<td>Mouse mammary epithelial 3-D cultures</td>
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<td>Human idiopathic pulmonary fibrosis fibroblasts</td>
<td>Rat neural progenitor cells</td>
<td>Field poppy pollen tubes</td>
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<td>Mouse immature B-cells</td>
<td>Mouse oligodendrocytes</td>
<td>Human, mouse retinal pigmented epithelial cells</td>
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Table 3. Primary cells tested with NucView 488 caspase-3 substrate

<table>
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<tr>
<th>Product description</th>
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<tr>
<td>NucView™ 488 Caspase-3 Assay Kit for live cells</td>
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<tr>
<td>Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™594-Annexin V</td>
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<tr>
<td>NucView™ 488 and MitoView™ 633 Apoptosis Kit</td>
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<tr>
<td>NucView™ 488 Caspase-3 Enzyme Substrate 1 mM in DMSO</td>
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<td>NucView™ 488 Caspase-3 Enzyme Substrate 1 mM in PBS</td>
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<tr>
<td>Ac-DEVD-CHO Caspase-3 Inhibitor, 5 mg</td>
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<td>Ac-DEVD-CHO Caspase-3 Inhibitor, 1 mg</td>
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<td>Ac-DEVD-AMC, 5 mg</td>
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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.
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.

**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

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**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.

**References**

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, 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).

<table>
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<tr>
<td>30067</td>
<td>NucView™ 488 Caspase-3 Substrate and CF™594-Annexin V Dual Apoptosis Assay Kit</td>
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<td>30065</td>
<td>Apoptosis &amp; Necrosis Quantitation Kit Plus</td>
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<td>30066</td>
<td>Apoptotic, Necrotic &amp; Healthy Cells Quantitation Kit Plus</td>
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<td>30060</td>
<td>CF™488A Annexin V and 7-AAD Apoptosis Kit</td>
</tr>
<tr>
<td>30061</td>
<td>CF™488A Annexin V and PI Apoptosis Kit</td>
</tr>
</tbody>
</table>

References
Internucleosomal cleavage of DNA is a hallmark of apoptosis. 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.

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.

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.
Calcein-AM Cell Viability Assay

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

MTT, XTT, and Resazurin 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 assaying cell viability and quantitating cell number. MTT and XTT are reduced to colored formazin salts that can be measured by absorbance. Resazurin 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.

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.

CFDA SE Cell Proliferation Dye is hydrolyzed in cells to release green fluorescent carboxy-fluorescein, for detection in the FITC channel.

References


www.biotium.com

Apoptosis, Necrosis and Cell Viability Assays
Vital Dyes, Apoptosis Inducers, Bacterial Viability

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

RedDot™1 and RedDot™2 are novel far red nuclear stains developed at Biotium. RedDot™1 is a live cell stain, 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.

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

**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).

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**Catalog number**

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<td>59007</td>
<td>Ionomycin, calcium salt</td>
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<td>30027</td>
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<td>32001</td>
<td>Bacterial Viability and Gram Stain Kit</td>
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<td>40019</td>
<td>PMA™ dye, 20 mM in water</td>
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</tbody>
</table>

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References

Additional Life Science Research Products

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
- VivoBrite™ Rapid Antibody Labeling kits for near-infrared small animal imaging

Genomics and proteomics products:

- GelRed™ and GelGreen™ non-toxic nucleic acid gel stains
- Environmentally safe EvaGreen® qPCR dye and master mixes
- AccuBlue™ DNA quantitation kits
- Lumitein™ fluorescent protein gel stain

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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