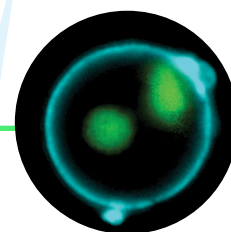


NucView™ 488 Caspase-3 Detection in Living Cells



NucView™ 488 Caspase-3 Substrate is a novel fluorescent probe that allows detection of caspase-3/7 activity in intact cells in real-time. Biotium scientists invented a novel fluorogenic enzyme substrate design by attaching an enzyme substrate moiety to a nucleic acid dye. In the case of NucView 488 Caspase-3 Substrate, the NucView 488 DNA dye is attached to the caspase-3/7 substrate peptide sequence DEVD. Once linked to the substrate peptide, the dye is unable to bind to DNA and remains non-fluorescent. The substrate crosses the plasma membrane to enter the cytoplasm, where it can be cleaved by caspase-3/7 to release the high-affinity DNA dye, which migrates to the cell nucleus to stain the nucleus with bright green fluorescence (Figure 1). Therefore, the substrate allows both detection of caspase-3 and visualization of nuclear morphology in apoptotic cells (Figure 3). Detection of caspase-3/7 activity using NucView 488 Caspase-3 Substrate has been reported for a wide variety of immortalized and primary cells in adherent, suspension and 3-D cultures (Table 1 and Table 2).

Unlike fluorescently-labeled caspase inhibitor assays (FLICA) that use irreversible inhibitors to label active caspases, NucView 488 Caspase-3 Substrate does not interfere with caspase activity, allowing monitoring of caspase activity in real time.

Biotium offers assay kits featuring NucView 488 Caspase-3 Substrate for the measurement of caspase-3/7 activity in combination with Annexin V staining, mitochondrial membrane potential, or vital dye staining by flow cytometry, microscopy, or fluorescence plate reader (see other side).

Reference: Cen, H., et al. FASEB J, 2008. 22 (7): p. 2243-52.

Key Features:

- **Caspase-3/7 dependent staining of DNA in intact cells**
- **Real-time monitoring of caspase-3/7 activity**
- **For use in adherent or suspension cells**
- **Rapid, homogenous, no-wash assay**
- **For flow cytometry, microscopy or microplate reader using FITC settings**
- **Formaldehyde-fixable**

Table 1. Cell lines tested with NucView 488 Caspase-3 Substrate

293-H	FU-UR-1	HUH6	MG-63	SKLMS1
293-T	GE11	Jurkat	Min 6	SW684
4T1	H9c2	JY	MOLT-3	SW872
67NR	HaCaT	K562	N19	TK6
A172	HCLE	LLC-PK1	NRK	U2OS
A204	HeLa	MCF-7	NRK-52E	U251
B16F10	HepT1	MCF-10A	PC-3	U373 MG
BeWo	HL-60	MDA-MB-231	PC12	WEHI 7.2
CCL-134	HMEC	MDCK	RD	
CCL-190	HOS	MES-SA	RINm5F	
CCRF-CEM	HT-1080	MES-SA/DX	Saos-2	

Email techsupport@biotium.com to request reference list.

Table 2. Primary cells tested with NucView 488 Caspase-3 Substrate

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

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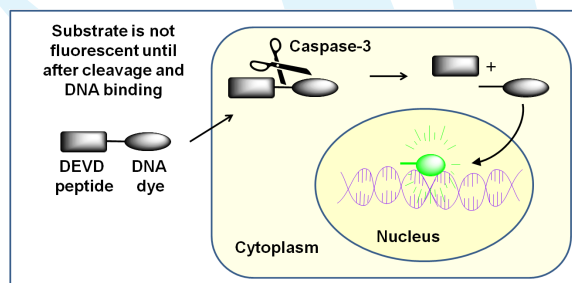


Figure 1. Schematic showing the principle of intracellular caspase-3/7 detection using NucView 488 Caspase-3 Substrate.

NucView™488 Caspase-3 Assay Kits

For detection by microscopy, flow cytometry, or fluorescence plate reader

NucView 488 Caspase-3 Assay Kit for Live Cells

Contains NucView 488 Caspase-3 substrate in DMSO and caspase-3/7 inhibitor Ac-DEVD-CHO.

NucView 488 and MitoView™ 633 Apoptosis Kit

Includes Biotium's proprietary far red fluorescent MitoView 633 mitochondrial membrane potential dye (Ex/Em 622/648 nm) for simultaneous measurement of caspase-3/7 activity and mitochondrial membrane potential (Figure 2).

Dual Apoptosis Assay with NucView 488 Caspase-3 Substrate and CF™594 Annexin V

Includes deep red fluorescent CF594 Annexin V (Ex/Em 593/614 nm) for dual detection of caspase-3 activity and phosphatidylserine translocation in intact cells (Figure 3). CF594 is brighter and more photostable than the spectrally similar dye Texas Red®.

NucView™488 and RedDot™2 Apoptosis & Necrosis 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 (Figure 4).

NucView™ 488 Caspase-3 Enzyme Substrate

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

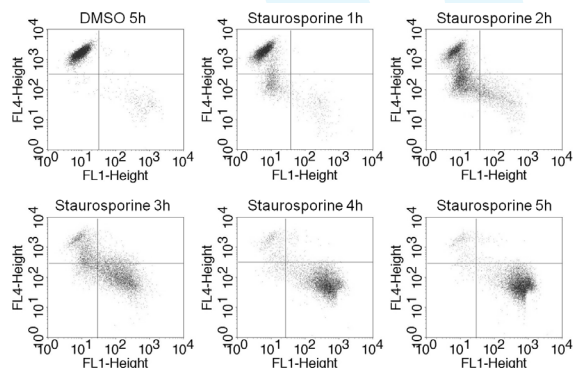


Figure 2. Flow cytometry analysis of caspase-3/7 activity and mitochondrial membrane potential in staurosporine-treated Jurkat cells using the NucView 488 and MitoView 633 Apoptosis Kit. NucView 488 staining is plotted on FL1 (x-axis) and MitoView 633 staining is plotted on FL4 (y-axis). As apoptosis progresses, NucView 488 signal increases while MitoView 633 signal decreases.

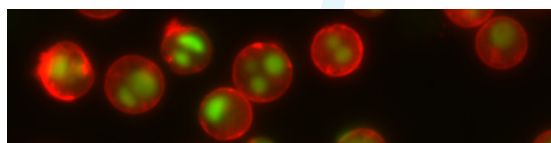


Figure 3. Jurkat cell stained with Dual Apoptosis Assay with NucView 488 Caspase-3 Substrate (green) and CF594 Annexin V (red) after induction of apoptosis with staurosporine.

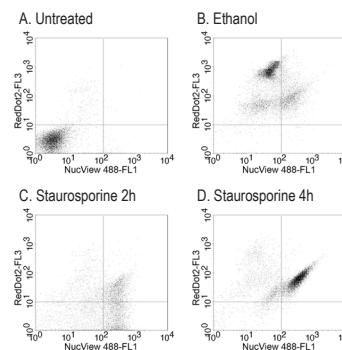


Figure 4. Flow cytometry analysis of Jurkat cells left untreated (A), treated with 10% ethanol for 90 minutes to induce necrosis (B), or treated with 1 μ M staurosporine for 2 hours (C) or 4 hours (D) to induce apoptosis. NucView 488 was detected in the FL1 channel (488 nm excitation and 530/30 nm emission 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.

Ordering Information

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 CF594™ Annexin V
30062	NucView™ 488 and MitoView™ 633 Apoptosis Kit
30072	NucView™ 488 and RedDot™2 Apoptosis and Necrosis Kit
10403	NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in PBS
10402	NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in DMSO

Visit www.biotium.com to learn more about our wide selection of fluorescence reagents for apoptosis and life science research.

Biotium products are for research use only. NucView enzyme substrate technology is covered by U.S. Patent No. 8,092,784.

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