Revised: November 28, 2017

Product Information

NucView® 488 and RedDot™2 Apoptosis & Necrosis Kit

Catalog Number: 30072

Unit Size: 100 assays based on flow cytometry protocol below.
The number of assays that can be performed may vary depending on the staining volume and reagent concentrations used.

Kit Contents
99925 NucView® 488 Caspase-3 Substrate, 0.2 mM in DMSO, 1 X 250 uL
40061-T RedDot™2, 200X in DMSO, 2 X 25 uL

Storage and Handling
Store at 4ºC. When stored as directed, the kit is stable for at least 6 months from the date it is received.

Spectral Properties
Absorption/emission maxima:
NucView® 488 bound to DNA: 500/530 nm (Fig. 1A)
RedDot™2 bound to DNA: 665/695 nm (Fig. 1B)

Product Description
NucView® 488 and RedDot™2 Apoptosis/Necrosis Assay Kit contains NucView® 488 Caspase-3 Substrate for detection of caspase-3/7 activity. The far-red dead-cell stain RedDot™2 is included for staining necrotic and late apoptotic cells that have compromised plasma membrane integrity. This kit provides a convenient tool for profiling apoptotic and necrotic cell populations by fluorescence microscopy or flow cytometry (Fig. 2).

In contrast to other fluorogenic caspase substrates or fluorescent caspase inhibitor based (FLICA) assays, NucView® 488 Caspase-3 Substrate can be used to detect caspase-3/7 activity within individual intact cells without inhibiting apoptosis progression. The substrate consists of a fluorogenic DNA dye coupled to the caspase-3/7 DEVD recognition sequence. The substrate, which is initially non-fluorescent, penetrates the plasma membrane and enters the cytoplasm. In apoptotic cells, caspase-3/7 cleaves the substrate, releasing the high-affinity DNA dye, which migrates to the cell nucleus and stains DNA with bright green fluorescence. Thus, NucView® 488 Caspase-3 Substrate allows detection caspase-3/7 activity and visualization of morphological changes in the nucleus during apoptosis.

RedDot™2 is a cell membrane-impermeable, far-red dye with high selectivity for membrane compromised or dead cells. The far-red emission of RedDot™2 is well-separated from the green fluorescence of NucView® 488. RedDot™2 can be excited by wavelengths from 488 to 647 nm, and therefore can be used with the 488 nm flow cytometry laser line.

Note: while NucView® 488 staining is formaldehyde-fixable and compatible with subsequent immunostaining, fixation after staining with RedDot™ 2 is not recommended because it can result in increased background staining of healthy cells.

References
Cen H, et al. FASEB J. 22, 2243–2252 (2008); Monaco, G., et al. Cell Death and Differentiation DOI 10.1038/cdd.2011.97 (2011); Schmitt, H., et al. Diabetologia DOI 10.1007/s00125-011-2133-5 (2011).

Please visit www.biotium.com to download a list of cell types tested with NucView® 488 Caspase-3 Substrate with references.

Assay protocols

Assay Optimization:
The protocols below were developed using Jurkat cells. Substrate and dye concentration can be varied to optimize the protocol for other cell types. Optimal NucView® concentration may vary between 1-5 uM for flow cytometry and 1-10 uM for fluorescence microscopy. Optimal RedDot™ 2 concentration may range from 0.25X to 1X. Cells can be incubated with substrate in culture medium, PBS, or other buffer of your choice. For adherent cells, we recommend removing medium and replacing with fresh medium containing substrate because high background can result in the area where concentrated substrate is added to the well. Media change or washing after incubation with substrate is not required.

For flow cytometry:

1. Induce apoptosis by desired methods. Remember to include an untreated cell sample as a negative control and a cell sample treated with a known inducer of apoptosis as a positive control.
2. For adherent cells, detach cells from culture substrate using trypsin or another cell dissociation method prior to staining.
3. Resuspend cells at a density of 10^6 cells/mL in culture medium or buffer.
4. Pipette 0.2 mL cell suspension into a flow cytometry test tube.
5. Add 2.5 uL of 0.2 mM NucView® 488 substrate stock solution to tube and immediately mix well to obtain a final NucView® 488 substrate concentration of 2.5 uM.
6. Dilute 200X RedDot™2 to 20X by mixing 1 uL of 200X RedDot™2 with 9 uL of buffer or medium. Add 2.5 uL of 20X (diluted) RedDot™2 to the tube and immediately mix well to obtain a final concentration of 0.25X.

   Note: alternatively, cells can be pelleted and resuspended in fresh medium or PBS containing 2.5 uM NucView® 488 substrate and .25X RedDot™2. For every 200 uL of staining solution required, add 2.5 uL of NucView® 488 substrate and 2.5 uL of 20X (diluted) RedDot™2.
7. Incubate cells at room temperature for 15-30 minutes, protected from light.
8. Add 300 uL medium or PBS to each tube and analyze by flow cytometry. Measure NucView® fluorescence in the green detection channel (excitation/emission: 485/515 nm). Measure RedDot™2 fluorescence in the far red channel (excitation/emission 665/695 nm). RedDot™2 also can be imaged in the PE-Cy5 or PerCP channel (488 nm excitation/far-red emission).

For fluorescence microscopy

1. Induce apoptosis by desired methods. Remember to include an untreated cell sample as a negative control and a cell sample treated with a known inducer of apoptosis as a positive control.
2. Prepare staining solution in cell culture medium or buffer. Dilute 200X RedDot™2 to 20X by mixing 1 uL of 200X RedDot™2 with 9 uL of buffer or medium. For every 200 uL of staining solution required, add 5 uL of NucView® 488 substrate and 5 uL of 20X (diluted) RedDot™2 for a final concentration of 5 uM NucView® 488 substrate and 0.5X RedDot™2.
3. Replace medium on cells with staining solution. Incubate cells with substrate at room temperature for 30 minutes or longer.
4. Image NucView® fluorescence using settings for settings for green fluorescence (FITC filter set, or excitation/emission 485/515 nm). Image RedDot™2 fluorescence using settings for far-red fluorescence (Cy5 filter set, or excitation/emission 665/695 nm. RedDot™2 can be excited by wavelengths from 488 nm to 647 nm.

   Optional: Cells can be counterstained with Hoechst 33342 dye (catalog number 40046) at a final concentration of 1 ug/mL to stain all cell nuclei with blue fluorescence (excitation/emission: 346/460 nm).

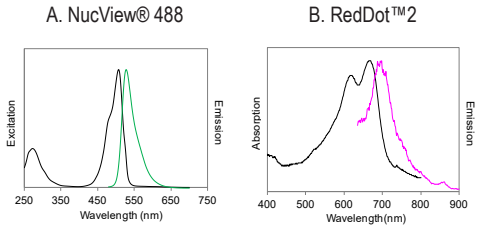

Figure 1. A. Excitation and emission spectra of enzymatically-cleaved NucView® 488 caspase-3 substrate with dsDNA. B. Absorption and emission spectra of RedDot™2 with dsDNA.

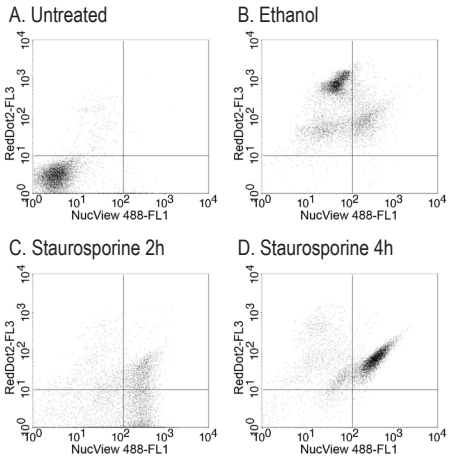

Figure 2. Flow cytometry analysis of Jurkat cells left untreated (A), treated with 10% ethanol for 90 minutes to induce necrosis (B), or treated with 1 uM staurosporine for 2 hours (C) or 4 hours (D) to induce apoptosis. Cells were stained for 30 minutes at room temperature with 2.5 uM NucView® 488 and 0.25X RedDot™2. 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.

Related Products

Catalog number	Description
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10407	NucView® 405 Caspase-3 Substrate, 1 mM in PBS
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10403	NucView® 488 Caspase-3 Substrate, 1 mM in PBS
10406	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO
10408	NucView® 530 Caspase-3 Substrate, 1 mM in PBS
30029	NucView® 488 Caspase-3 Assay Kit for Live Cells
30067	Dual Apoptosis Assay Kit with NucView® 488 caspase-3 substrate & CF®594-Annexin V
30062	NucView® 488 and MitoView™ 633 Apoptosis 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
30001	JC-1 Mitochondrial Membrane Detection Kit
70055	MitoView™ 633 mitochondrial membrane potential dye
32002-32010	Live-or-Dye™ Fixable Viability Staining Kits
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO

Please visit our website at **www.biotium.com** to view our full selection of products for cell viability and apoptosis detection,along with hundreds of other products for cell biology, genomics, and proteomics research.

Frequently Asked Questions

Question	Answer
How stable is NucView® 488 Caspase-3 Substrate?	The substrate is very stable. Some users have reported performing time course assays with NucView® 488 Caspase-3 Substrate for 4-5 days at 37°C.
When should I add NucView® 488 Caspase-3 Substrate to my cells?	NucView® 488 Caspase-3 Substrate can be added to the cells at the start of the experiment or at the end. NucView® 488 Caspase-3 substrate does not affect the time course of apoptosis progression, so a major advantage of NucView® 488 Caspase-3 Substrate compared to other apoptosis assays is that it can be used to monitor caspase-3 activity in real time.
Can the NucView® 488 and RedDot2 Apoptosis & Necrosis Kit be used for tissue staining?	The assay cannot be used in fixed cells or tissue sections. The assay has not been validated by Biotium for live tissue staining.
Can I fix NucView® 488 Caspase-3 Substrate for subsequent immunostaining?	NucView® 488 is formaldehyde fixable, however, fixation of RedDot2 after staining may increase background staining of healthy cells and is not recommended.
How long can I monitor NucView® 488 Caspase-3 Substrate under the microscope?	As with other fluorescence based probes, photobleaching may during imaging. How long you can view NucView® 488 staining under the microscope depends on several factors including the initial signal strength and the intensity of the excitation source.
How specific is NucView® 488 Caspase-3 Substrate for caspase-3?	Like other caspase-3 substrates, NucView® Caspase-3 Substrates are based on a DEVD caspase-3 consensus sequence that also can be cleaved by caspase-7. Other caspases may also cleave DEVD substrates due to overlapping substrate specificity among caspases.
What cell types can be used with NucView® 488 Caspase-3 Substrate?	NucView® 488 Caspase-3 Substrate has been reported to work in a wide variety of primary cells and immortalized cell lines in the published scientific literature. Visit www.biotium.com to download a list of cell types and references.