Revised: November 28, 2017

Product Information

NucView® 488 and MitoView™ 633 Apoptosis Assay Kit

Catalog Number: 30062

Unit Size: 100 assays (based on 200 uL sample volume)

Kit Contents

Component	Size
99949: NucView® 488 Caspase-3 Substrate, 200X in DMSO	100 uL
99950: MitoView™ 633, 200X in DMSO	100 uL

Storage and Handling

Store at 4C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties

NucView® 488: Ex/Em 500/530 nm (free dye bound to DNA)
MitoView™ 633: Ex/Em 622/648 nm
See Figure 1.

Product Description

This kit contains NucView® 488 Caspase-3 Substrate and MitoView™ 633 mitochondrial membrane potential dye. The kit provides a convenient tool for profiling apoptotic cells based on caspase-3/7 activity and changes in the mitochondrial membrane potential using either flow cytometry (Figure 2) or fluorescence microscopy (Figure 3). The spectral separation of the two dyes minimizes fluorescence overlap (Figure 1).

Note: The optimal detection settings for MitoView™ 633 are the same as for Cy®5 and other far-red dyes. However, the dye also has visible red fluorescence emission (see Figure 1) and can be imaged using Cy®3 settings as well. As a consequence, the dye cannot be used for two-color imaging with other red probes.

Unlike conventional caspase assays, NucView® 488 Caspase-3 substrate detects caspase-3/7 activity within individual intact cells without inhibiting apoptosis. The substrate consists of a fluorogenic DNA dye and a DEVD substrate moiety. The substrate, which is both non-fluorescent and nonfunctional as a DNA dye, crosses the plasma membrane to enter the cytoplasm, where it can be cleaved by caspase-3/7 to release a high-affinity DNA dye. The free dye migrates to the nucleus to stain DNA with bright green fluorescence. NucView® 488 Caspase-3 Substrate is bi-functional, allowing detection of caspase-3/7 activity and also visualization of changes in nuclear morphology during apoptosis (Figure 3). NucView® 488 detection of apoptosis has been reported in a wide variety of immortalized and primary cell types in peer reviewed scientific publications. Visit www.biotium.com for more information, including FAQs and reference lists.

MitoView™ 633 is a far-red fluorescent mitochondrial dye. MitoView™ 633 is cell membrane permeable and becomes brightly fluorescent after accumulating in mitochondria. The staining is dependent upon the mitochondrial membrane potential; thus, apoptotic cells that have lost mitochondrial membrane potential show much lower MitoView™ 633 staining than healthy cells (Figure 2 and Figure 3).

Assay Protocol

1. Prepare pre-warmed staining medium containing 1X NucView® 488 and 1X MitoView™ 633. For example, add 1 uL of 200X NucView® 488 and 1 uL of 200X MitoView™ 633 to 200 uL of cell culture medium and mix well. Also see note under step 2.

   Note: For larger sample volumes, scale all components proportionally.

   Note: The optimal concentration of NucView® 488 and MitoView™ 633 may vary by application and cell type.

2. Remove the culture medium from your cells and replace with staining medium. For suspension cells, pellet the cells and resuspend in staining medium.

   Note: alternatively, the dye can be added directly to the cells in their current culture medium. We recommend diluting the 200X DMSO stocks in culture medium before adding to the cells to avoid transient, localized high dye concentrations. For example, add 1 uL of 200X NucView® 488 Caspase-3 Substrate and 1 uL of 200X MitoView™ 633 to 20 uL of culture medium, then add the entire volume to your cells in 200 uL of culture medium, and mix well by gently pipetting up and down. For larger sample volumes, scale all components proportionally.

3. Incubate cells for 15 minutes or longer at 37°C.

   Note: NucView® and MitoView™ can be used for extended incubation times for real-time imaging, ranging from hours to days (see Figure 2).

   Optional: you can replace the staining solution with fresh medium or buffer prior to imaging. For suspension cells, pellet the cells and resuspend in fresh medium or buffer.

4. Analyze fluorescence by fluorescence microscopy or flow cytometry using 488 nm excitation and filter sets for FITC for NucView® 488, and 633 nm excitation and filter sets for Cy®5. Alternatively, MitoView™ 633 can be imaged using 555 nm excitation and filter sets for Cy®3 (see note under product description).

   Note: If cells are not stained sufficiently, increase the dye concentration or the staining time.

   Note: Cells stained with NucView® 488 can be fixed in formaldehyde, however, fixation will abolish MitoView™ 633 staining.

   Note: For flow cytometry acquisition, position the healthy cell population within the first decade in the FITC channel (NucView® staining) and within the upper two decades in the Cy®5 channel (MitoView™ staining) (see Figure 2).

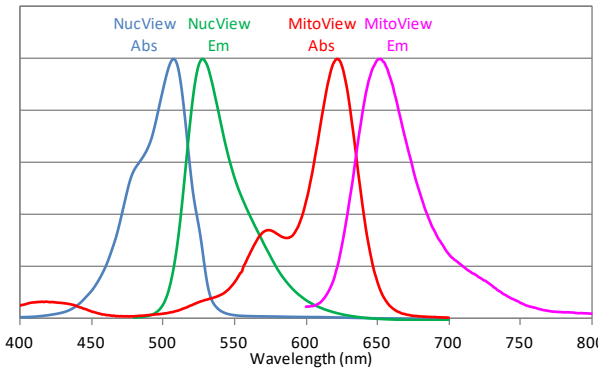

Figure 1. Left: normalized excitation and emission spectra of NucView® 488 dye after substrate cleavage in the presence of DNA. Right: normalized absorbance and emission spectra of MitoView™ 633 in methanol.

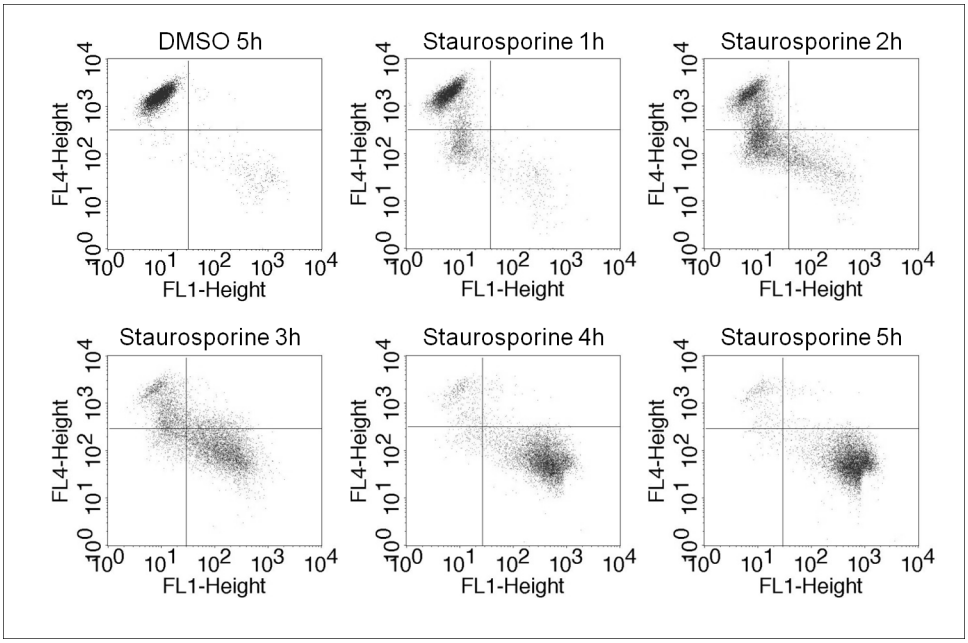

Figure 2: Flow cytometry analysis of apoptosis using NucView® 488 Caspase-3 Substrate and MitoView™ 633 mitochondrial membrane potential dye. Jurkat cells were incubated with NucView® 488 and MitoView™ 633 and with or without staurosporine for 5 hours. Cells were analyzed on a FACSCalibur (BD BioSciences) at 1 hour intervals. NucView® 488 fluorescence was detected in the FL1 channel while MitoView™ 633 fluorescence was detected in the FL4 channel. Control cells were treated with DMSO (vehicle) for 5 hours.

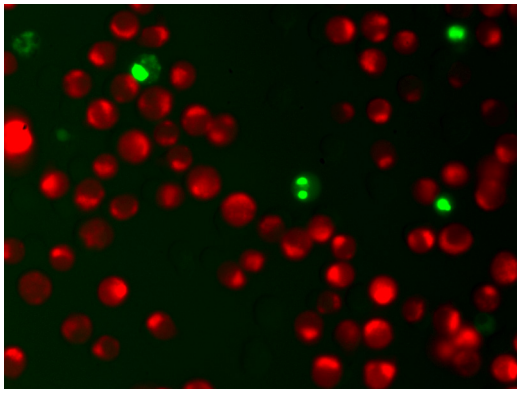

Figure 3. Fluorescence microscopy of Jurkat cells stained with NucView® 488 and MitoView™ 633. Healthy cells stain with MitoView™ 633 (red) while late apoptotic cells with fragmented nuclei strongly stain with NucView® 488 (green), but show low or undetectable staining with MitoView™ 633 due to loss of mitochondrial membrane potential.

Related Products

Catalog number	Product
70070	MitoView™ 405
70054	MitoView™ Green
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10403	NucView® 488 Caspase-3 Substrate, 1 mM in PBS
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10407	NucView® 405 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
30067	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®594 Annexin V
30073	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®640R Annexin V
30072	NucView® 488 and RedDot™ 2 Apoptosis & Necrosis Kit
30029	NucView® 488 Caspase-3 Assay Kit for live cells
10405	NucView® 405 Caspase-3 Substrate
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
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
70058	LysoView™ 633

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