Apoptosis Assay Kit
NucView™ 488 and MitoView™ 633

Catalog Number: 30062 (100 Assays)

Revised: May 2, 2012

Contact Information

www.interchim.com

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com

Description

The kit contains the NucView™ 488 Caspase-3 Substrate and the MitoView™ 633 mitochondrial 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 fluorescence microscopy or flow cytometry. Furthermore, the spectral separation of these two dyes minimizes any fluorescence overlap.

Different from the conventional caspase assays, NucView™ 488 Caspase-3 substrate detects caspase-3/7 activity within individual intact cells in a non-interfering manner. 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, rapidly crosses cell membranes to enter the cell cytoplasm, where it is cleaved by caspase-3/7 to form a high-affinity DNA dye. The released DNA dye migrates to the cell nucleus to stain the nucleus bright green. Thus, the NucView™ 488 Caspase-3 Substrate is bi-functional, being able to detect both intracellular caspase-3/7 and at the same time stain the cell nucleus, which is known to undergo morphological changes during apoptosis.

MitoView™ 633 is a far-red fluorescent mitochondrial dye. The cell permeable MitoView™ 633 reagent diffuses across the plasma membrane and accumulates in the mitochondria. Mitochondria stained with MitoView 633 become brightly fluorescent after accumulation of the dye in the lipid environment of mitochondria. The staining is dependent upon the mitochondrial membrane potential; thus, apoptotic cells exhibit a much lower MitoView ™ 633 dye staining compared to healthy cells.

The concentration of NucView ™ 488 and MitoView ™ 633 for optimal staining may vary by application and cell type. The recommended procedure below is a general guideline and may need to be optimized. Our protocol is designed for 200 µL sample volumes using NucView ™ 488 and MitoView ™ 633. In some cases, the optimal working concentrations of NucView ™ 488 and MitoView ™ 633 may need to be determined empirically. At too high a concentration, these probes may stain other cellular structures.

Kit Components

1 vial (100 µL); NucView™ 488 Caspase-3 substrate, 200X in DMSO
1 vial (100 µL); MitoView ™ 633 Mitochondrial Dye, 200X in DMSO

Storage Condition

NucView™ 488 Live Cell Caspase-3 Assay Kit should be stored at 4°C. The components of the kit are stable at 4°C for at least six months.

Spectral Characteristics

Abs/Em maxima: NucView™ 488: 500/530 nm (bound to DNA)
MitoView™ 633: 622/648 nm

Procedure

Staining of adherent cells:

1. Grow cells on coverslips in a dish or directly onto dish if slide mounting is not desired.
2. When cells are at appropriate confluency, remove the medium and add 200 µL fresh prewarmed medium containing 1 µL NucView™ 488 and 1 µL MitoView™ 633. Alternatively, the probes can be added directly to the current culture medium.
3. Incubate cells for 30 minutes (or longer).
4. Replace the loading solution with fresh medium or PBS and observe cells using a fluorescence microscope. Washes are not necessary, but may result in higher background.

Staining of suspension cells:

1. Pellet cells and aspirate the supernatant.
2. Resuspend pellet in 200 µL medium containing 1 µL NucView™ 488 and 1 µL MitoView™ 633. Alternatively, the probes can be added directly to the current culture medium.
3. Incubate for 30 minutes (or longer).
4. Centrifuge the cells and resuspend pellet in fresh medium or PBS and observe cells using a fluorescence microscope or flow cytometer. Washes are not necessary, but may result in higher background.

Note: If cells are not stained sufficiently, increase the concentration or the incubation time for the dyes to accumulate in the nucleus and mitochondria. In some cases, the optimal final concentration may need to be determined empirically. Subsequent fixation and permeabilization steps will affect MitoView™ 633 staining.

If a control for caspase-3 specificity of NucView™488 staining is desired, we recommend using a caspase-3 inhibitor such as Z-DEVD-FMK along with the apoptosis inducer.

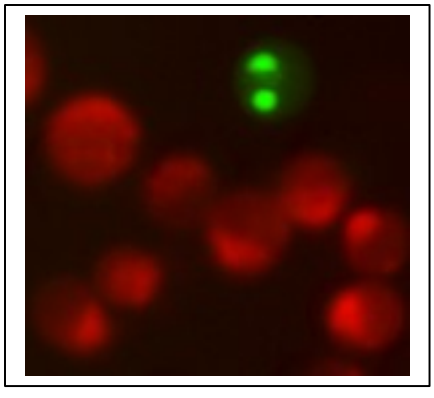

Figure 1: Microscopy of Jurkat cells stained with NucView™ 488 and MitoView™ 633. Healthy cells are strongly stained with MitoView™ 633 (red) while the apoptotic cell with fragmented nucleus is strongly stained with NucView™ 488 (green). There is an inverse correlation between these probes.

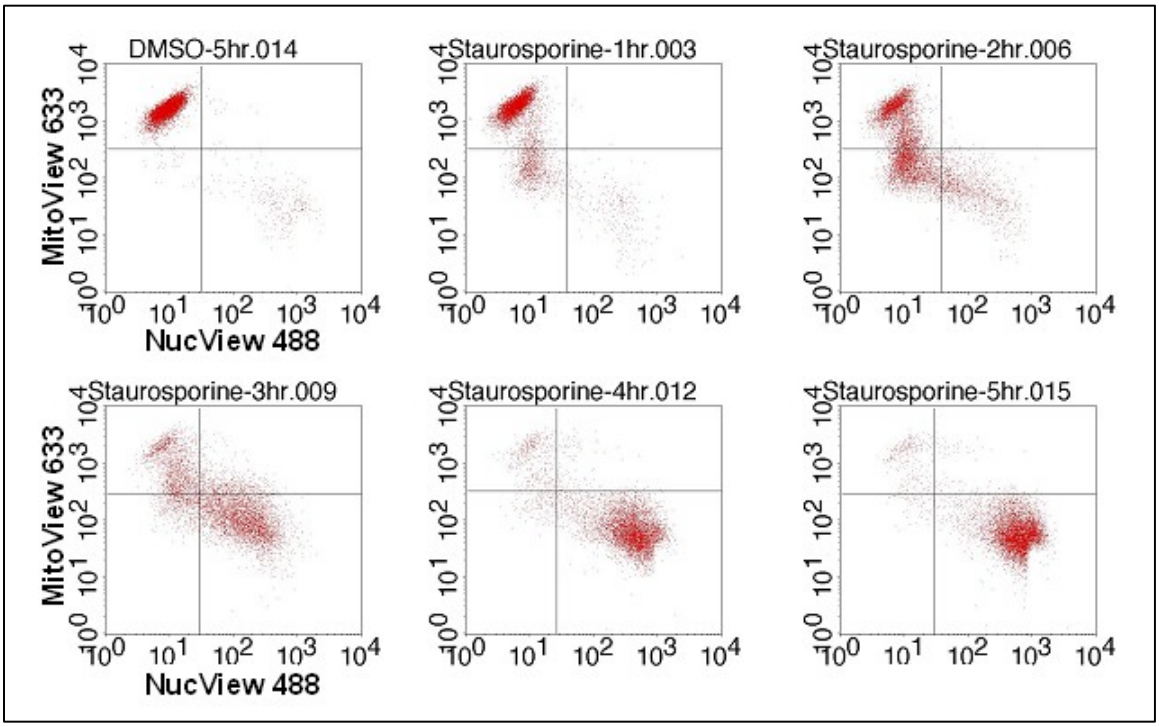

Figure 2: Jurkat cells were incubated with NucView™ 488 and MitoView™ 633 and with or without staurosporine for 5 hours. Cells were analyzed on a FACSCalibur (BD BioSciences) at 1 hr intervals. NucView™ 488 fluorescence was detected in the FL1 channel while MitoView™ 633 fluorescence was detected in the FL4 channel. Control cells were treated with DMSO (vehicle) for 5 hours.

Note: For flow cytometry acquisition and analysis, position healthy cells with NucView™ 488 staining within the first decade and MitoView™ 633 staining within the upper two decades.