



NucView™ 488 Caspase-3 Substrate for Live Cells (1 mM in 1X PBS)

Catalog Number: 10400

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Description

NucView™ 488 Caspase-3 substrate is a novel cell membrane-permeable fluorogenic caspase substrate designed for detecting caspase-3 activity within live cells in real time.

The rate of apoptosis typically varies from cell to cell even within the same population. As a result, various apoptotic events or markers accompanying the apoptotic process also occur differently among cells. Thus, it is important to be able to detect these apoptotic events on an individual cell basis. Traditionally, caspase activity has been detected either using a membrane-impermeable fluorogenic enzyme substrate such as DEVD-R110, or a fluorescently-labeled inhibitor such as a FLICA reagent. In the former case, cell lysis is required, thus precluding the detection of caspase activity in live cells. In addition, such caspase assays measure only the average caspase activity of a highly heterogeneous cell population at a given time. In the latter case, although a FLICA reagent can enter live cells to detect caspase activity, only the initial fluorescent signal following the application of the reagent can truly reflect the enzyme activity or the state of the apoptotic cells because any detected signal after the initial “snap shot” will need to consider the potential interference of the inhibitor to the enzyme and the apoptotic cell itself.

Different from the conventional caspase assays, NucView™ 488 Caspase-3 substrate detects caspase-3 activity within individual intact cells in a noninterfering manner. The substrate consists of a fluorogenic DNA dye and a DEVD substrate moiety specific for caspase-3. The substrate, which is both nonfluorescent and nonfunctional as a DNA dye, rapidly crosses cell membrane to enter the cell 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 bright green. Thus, the NucView™ 488 caspase-3 substrate is bi-functional, being able to detect both intracellular caspase-3 and at the same time stain the cell nucleus, which is known to undergo morphological change during the apoptosis process. The fluorescent staining produced in response to caspase-3 activity is fixable via standard fixation method (3.75% formaldehyde in PBS for fixation, 0.5% Triton-X 100 in PBS for cell permeabilization), thus facilitating any subsequent immunostaining study.

Storage Condition

NucView™ 488 Live Cell Caspase-3 Substrate is stable at 4°C for at least six months.

Features

Applicable to live cells: Detecting caspase-3 activity within individual live or dead cells in a cell population.

Bi-functional: being able to both detect caspase-3 activity and stain cell nuclei at the same time.

Simple & Fast: requiring only a 15-minute incubation time without washing for caspase-3-positive cells to be reliably detected.

Versatile: compatible with either flow cytometry for cell sorting-based analysis, or with fluorescence microscopy for following the fluorescence signal and morphological changes of cell nuclei in real-time using the fluorescein filter set.

Fixable: fluorescent image is fixable via a standard fixation (3.75% formaldehyde in PBS for fixation, 0.5% Triton-X 100 in PBS for cell permeabilization) method, thus permitting further immunostaining studies.

Excitation/Emission Spectra of NucView 488 Fluorescent Product

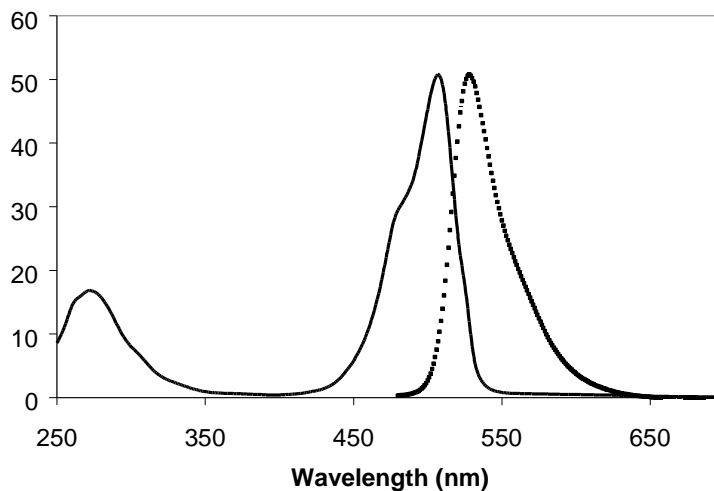


Figure 1: Excitation and emission spectra of enzymatically-cleaved NucView 488 caspase-3 substrate in the presence of excess of dsDNA.

Protocol

For detailed application protocol, please download the protocol for the ready-to-use NucView™ 488 Caspase-3 Assay Kit for live cells (cat#30029) :

http://www.biotium.com/product/product_info/Protocol/30029.pdf

Please note that the concentration of the substrate is **1 mM** in 1X PBS (not 0.2 mM as in the NucView™ 488 Caspase-3 Assay Kit). Please adjust the dilution accordingly.

Related Products

In addition to the substrate alone, we offer two ready-to-use assay kits with detailed application protocol:

1) NucView™ 488 Caspase-3 Assay Kit for live cells (cat#30029)

download protocol: http://www.biotium.com/product/product_info/Protocol/30029.pdf

2) Dual Apoptosis Assay with NucView™ 488 caspase-3 substrate and Sulforhodamine 101-Annexin V (cat#30030)

download protocol: http://www.biotium.com/product/product_info/Protocol/30030.pdf

Reference:

1) Benetti, L, *et al. J. of Virology*, 10242–10248 (Oct.2007); 2) Leuenroth, SJ, *et al. PNAS*, **104** (11), 4389-4394 (2007); 3) Tribulatti, M, *et.al. Glycobiology*, **17**(11), (2007)