DATA SHEET

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

Catalog Number: 10403

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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 without inhibiting caspase-3 activity. The substrate consists of a fluorogenic DNA dye and a DEVD substrate moiety specific for caspase-3. The substrate, which is both non-fluorescent and nonfunctional as a DNA dye, rapidly crosses cell membranes to enter the cytoplasm, where it is cleaved by caspase-3 to form a high-affinity DNA dye. The released DNA dye stains the nucleus bright green. Thus NucView™ 488 caspase-3 substrate is bi-functional, allowing detection of caspase-3 activity and at the same time staining the dying cell nucleus, which undergoes morphological changes during apoptosis. The fluorescent staining produced in response to caspase-3 activity is fixable via standard fixation and permeabilization methods for subsequent immunostaining (3.75% formaldehyde in PBS for fixation, 0.5% Triton-X 100 in PBS for cell permeabilization).

Storage and Handling

NucView™ 488 Live Cell Caspase-3 Substrate is stable at 4°C for at least six months from the date of receipt. Protect from light.

Features

Live cell caspase-3 assay: Detect caspase-3 activity within individual apoptotic cells in a cell population.
Bi-functional: Detect caspase-3 activity and observe apoptotic nuclear morphology simultaneously.
Simple & Fast: Requires only a 15-minute incubation time without washing for caspase-3-positive cells to be reliably detected.
Versatile: Compatible with flow cytometry, fluorescence microscopy, or fluorescence microplate reader using fluorescein detection settings.
Fixable: Staining is compatible with standard fixation and permeabilization conditions for immunostaining (3.75% formaldehyde in PBS for fixation, 0.5% Triton-X 100 in PBS for permeabilization).

Excitation/Emission Spectra of NucView 488 Fluorescent Product

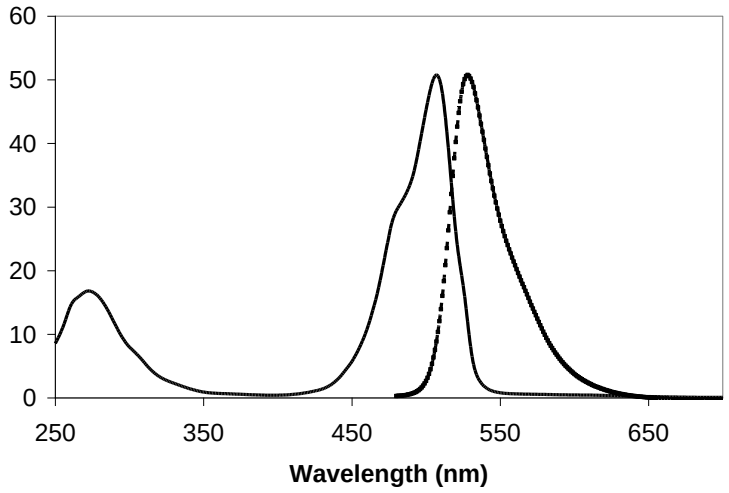

Figure 1: Excitation and emission spectra of enzymatically-cleaved NucView 488 caspase-3 substrate in the presence of excess of dsDNA.

References

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)

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