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Biotium, Inc.
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NucView™ 488 Caspase-3 Assay Kit for Live Cells

Catalog Number: 30029 (100-500 assays)

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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.

The kit contains both NucView™ 488 Caspase-3 substrate and caspase-3 inhibitor Ac-DEVD-CHO. The kit provides a convenient tool for profiling apoptotic cell population based on caspase-3 activity using either fluorescence microscopy or flow cytometry.

Kit Components

2 vials (250 uL/vial); NucView™ 488 Caspase-3 substrate, 0.2 mM in DMSO
1 vial (20 uL/vial); Caspase-3 inhibitor Ac-DEVD-CHO, 2 mM 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.

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, with microplate readers, 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

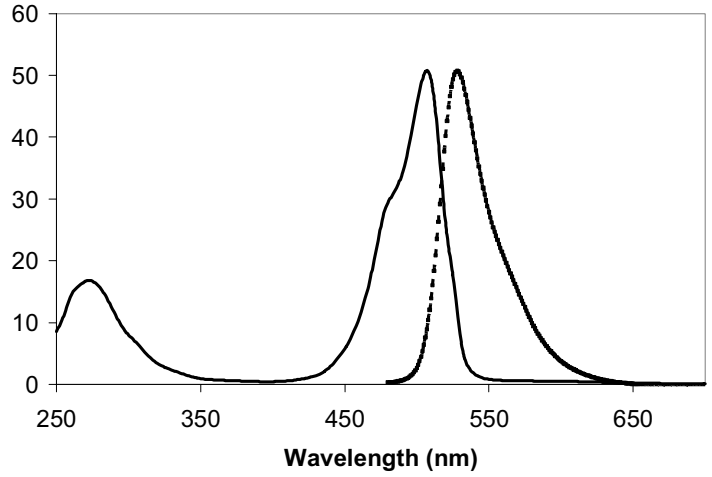

Figure 1: Excitation and emission spectra of enzymatically-cleaved NucView 488 caspase-3 substrate in the presence of excess of dsDNA.

Detection of Caspase-3 Activity in Live Cells

A. General Considerations

We recommend performing three control reactions:
1. Negative control on uninduced cells
2. Control on induced cells treated with Caspase-3 inhibitor (we recommend that you use a concentration 2-5 fold of the substrate and incubate the cells for 15-30 minutes prior to substrate addition)
3. Positive control for Caspase-3 induction

B. Assay Procedure for Flow Cytometry

1. Induce apoptosis in cells by desired methods. Remember to incubate concurrent culture without induction.

2. Resuspend cells in culture medium with appropriate concentration.
<u>For suspension cells:</u>
   a) Count cells and resuspend cells in culture medium or PBS into 0.5-1x10^6 cells/mL.

<u>For adherent cells:</u>
   a) Detach cells from the bottom of culture wells/plates by trypsin.
   b) Add an equal volume of cell culture medium with serum (as trypsin inhibitor) into trypsinized cells and resuspend cells thoroughly in the mixed solution into 0.5-1x10^6 cells/mL.

3. Pipette 0.2 mL cell suspension into a flow cytometry tube.
   Note: For testing specificity of NucView™ 488, add caspase-3 inhibitor Ac-DEVD-CHO in the next step and incubate the inhibitor at room temperature for at least 15 min. The final concentration of the inhibitor should be at least 2 times of the final concentration of NucView™ 488. For instance, if the final concentration of NucView™ 488 is 5 uM (5uL of 0.2 mM NucView™ 488 per sample shown below), the final concentration of Ac-DEVD-CHO should be at least 10 uM (1 uL of 2 mM Ac-DEVD-CHO).

4. Add 5 uL of 0.2 mM NucView™ 488 Caspase-3 substrate stock solution directly into cell suspension and mix well.
   Note: Optimal substrate concentration varies among cell lines. For optimization, use 1 to 10 uL of substrate stock solution to derive 1 to 10 uM final substrate concentrations.

5. Incubate at room temperature for 15-30 min minutes.

6. Add 0.3 mL medium or PBS and analyze in a flow cytometer using FL1 channel.
   Note: For end-point analysis, wash substrate away at your desired time point and resuspend cells in 500 uL medium or PBS before analyzing by flow cytometry. For kinetic analysis, increase the volume of cell suspension and substrate proportionally based on the number of your time points, skip adding 0.3 mL medium or PBS and directly proceed to flow cytometry analysis at your desired time points.

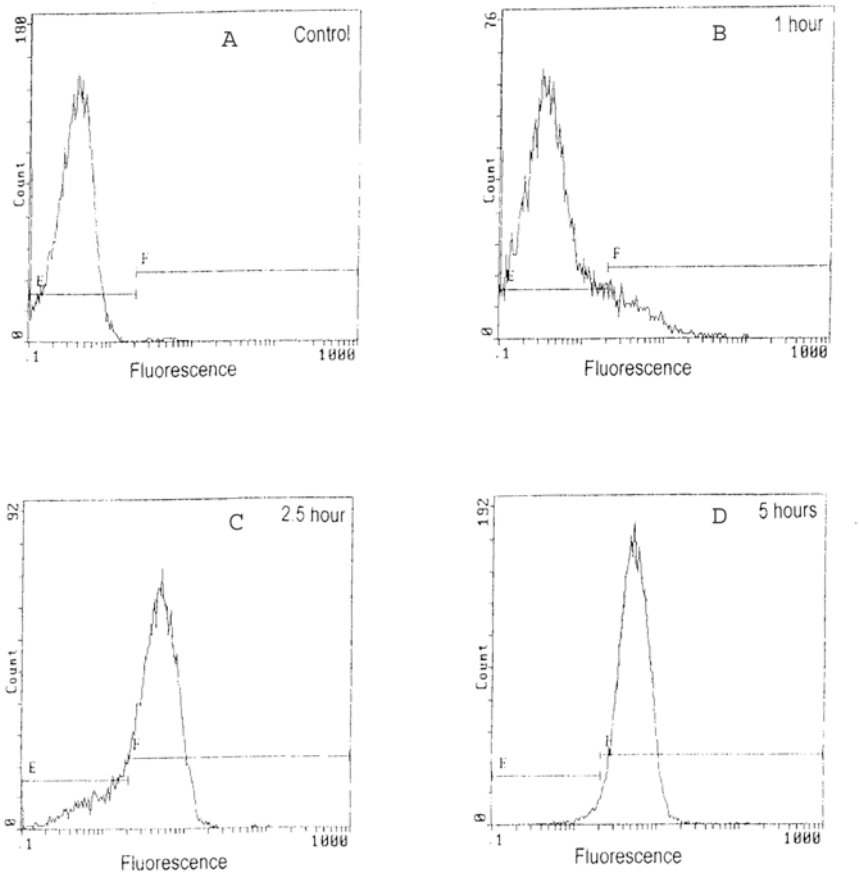

Figure 2: Detection of caspase-3 activated cells by flow cytometry in apoptotic Jurkat cell population. A flask of Jurkat cells were induced with 1 uM staurosporine for apoptosis. Aliquot of Jurkat cells were taken at 1 hr, 2.5 hrs and 5 hrs after induction together with an aliquot of uninduced Jurkat cells and assayed by NucView™ 488 Caspase-3 substrate according to the procedure described above. The data was obtained by using flow cytometer EPICS XL-MCL (Coulter, CA) using FL1 channel for green fluorescence.

C. Assay Procedure for Microscopy

1. Induce apoptosis in cells by desired methods. Remember to incubate concurrent culture without induction.

2. Assaying

<u>For suspension cells:</u>

a) Count cells and resuspend cells in PBS into 0.5-1x10^6 cells/mL.
b) Pippete 0.2 mL cell suspension into a tube.

Note: For testing specificity of NucView™ 488, add caspase-3 inhibitor Ac-DEVD-CHO in the next step and incubate the inhibitor at room temperature for at least 15 min. The final concentration of the inhibitor should be at least 2 times of the final concentration of NucView™ 488. For instance, if the final

concentration of NucView™ 488 is 5 uM (5uL of 0.2 mM NucView™ 488 per sample shown below), the final concentration of Ac-DEVD-CHO should be at least 10 uM (1 uL of 2 mM Ac-DEVD-CHO per sample).

c) Add 5 uL of 0.2 mM NucView™ 488 Caspase-3 substrate stock solution directly into cell suspension.

Note: Optimal substrate concentration varies among cell lines. For optimization, use 1 to 10 uL of substrate stock to derive 1 to 10 uM final substrate concentration.

d) Incubate at room temperature for 30min or longer.
e) Centrifuge cells in a microfuge tube at 4000rpm for 5 minutes.
f) Pipette out supernatant and resuspend cell pellet in 20uL PBS.

Note 1: For kinetic analysis, pipette out 90% of supernatant and resuspend cells in residual 20 uL staining solution.
Note2: For visualization of healthy cells, 2 uL of 10X DAPI solution (100 uM) is added into 20 uL cell suspension in step f) to reach 10 uM final concentration for DAPI. With the presence of DAPI, healthy cells are stained blue only while apoptotic cells are stained green and blue.

g) Mount cells into a slide and seal the slide.
h) Observe cell staining under a fluorescence microscope using FITC filter within 2 hours.

Note: For long-term storage of images, fix the NucView™ 488 stained cells with 3.75% formaldehyde before mounting and store slides at –20°C.

For adherent cells:

a) Grow cells on coverslips or chamber slides.
b) Prepare each cell sample in 200 uL PBS or grow medium.

Note: For testing specificity of NucView™ 488, add caspase-3 inhibitor Ac-DEVD-CHO in the next step and incubate the inhibitor at room temperature for at least 15 min. The final concentration of the inhibitor should be at least 2 times of the final concentration of NucView™ 488. For instance, if the final concentration of NucView™ 488 is 5 uM (5uL of 0.2 mM NucView™ 488 per sample shown below), the final concentration of Ac-DEVD-CHO should be at least 10 uM (1 uL of 2 mM Ac-DEVD-CHO per sample).

c) Add 5 uL 0.2 mM NucView™ substrate stock solution into 200 uL PBS or medium.

Note: Optimal substrate concentration varies among cell lines. For optimization, use 1 to 10 uL of substrate stock solution to derive 1 to 10 uM final substrate concentration.

d) Aspirate medium from the coverslip or chamber slide well.
e) Add appropriate volume of NucView™ 488 Caspase-3 substrate solution onto the coverslip or chamber slide well.

Note: NucView™ 488 may be directly added into existing culture medium as well.

f) Incubate at room temperature for 30 minutes or longer.
g) Mount the coverslip or chamber slide in the substrate-containing solution into a slide and seal the slide.

Note: For visualization of healthy cells, DAPI (10 uM as final concentration) can be added at this step. With the presence of DAPI, healthy cells are stained blue only while apoptotic cells are stained green and blue.

h) Observe cell staining under a fluorescence microscope using FITC filter at various time points to determine the optimal substrate staining time.

Note1: For end-point analysis, wash substrate away at the optimal substrate staining time identified above and mount the coverslip or chamber slide in PBS into a slide. Removal of substrate in mounting solution provides much better background.
Note2: For long-term storage of images, fix the NucView™ 488 stained cells with 3.75% formaldehyde before mounting and store slides at –20°C.

D. Assay Procedure for Microplate Readers (Suspension Cells)

1. Induce apoptosis in cells by desired methods. Remember to incubate concurrent culture without induction.

2. Cells can be treated in dishes or flasks and then aliquoted into black 96-well plates or cultured directly into wells and induced. Resuspend cells in culture medium with desired concentration of cells. 1 X 10^6 cells/mL is a good starting point but should be optimized as cell type, concentration of substrate, and sensitivity of the microplate reader can affect the readings.

3. Pipette 0.2 mL cell suspension into each well of a black 96-well plate.
   Note: For testing specificity of NucView™ 488, add caspase-3 inhibitor Ac-DEVD-CHO in the next step and incubate the inhibitor at room temperature for at least 15 min. The final concentration of the inhibitor should be at least 2 times of the final concentration of NucView™ 488. For instance, if the final concentration of NucView™ 488 is 5 uM (5uL of 0.2 mM NucView™ 488 per sample shown below), the final concentration of Ac-DEVD-CHO should be at least 10 uM (1 uL of 2 mM Ac-DEVD-CHO).

4. Add 5 uL of 0.2 mM NucView™ 488 Caspase-3 substrate stock solution directly into cell suspension and mix well. Incubate for 15-30 minutes.
   Note: Optimal substrate concentration varies among cell lines. For optimization, use 1 to 10 uL of substrate stock solution in 0.2 mL to derive 1 to 10 uM final substrate concentrations.

5. Read on plate reader at 488 nm excitation and 520 nm emission cut-off. These wavelengths can be adjusted depending on the specific plate reader and sensitivity necessary to optimize protocol.

Note: Although adherent cells can be used with NucView488 in microplate format, please be aware that the variability in areas of adherence from well to well can result in erratic readings.

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