NucView® 488 Caspase-3 Substrate

Catalog# / Size 421928 / 100 µL

Regulatory Status RUO

Other Names Caspase-3 substrate, Caspase 3, Caspase probe

Description Caspase 3 is an effector caspase that plays a major role in the apoptotic pathway and activated by initiator caspase during the early stages of apoptosis. Its activity ultimately leads to apoptotic chromatin condensation and DNA fragmentation.

This substrate is a four-amino acid DEVD peptide conjugated to a high-affinity DNA-binding dye. The non-fluorescent caspase-3 substrate enters the cytoplasm, and active Caspase-3 in apoptotic cells cleaves the substrate, releasing the DNA-binding dye. The DNA dye migrates to the nucleus and fluoresces upon binding to DNA. Thus, this probe allows for detection of caspase 3 activity in live cells by imaging and flow cytometry.

Product Details

Verified Reactivity Human

Reported Reactivity Mouse, Species independent

Formulation 1 vial of 100 µL of NucView® 488 Caspase-3 Substrate in DMSO

Storage & Handling 4°C; protected from light

Application Live cell imaging - Quality tested
FC - Verified

Recommended Usage Refer to the detailed protocol in the application notes section.

Application Notes Component:

- 100 µL of NucView® 488 Caspase-3 Substrate in DMSO (1 mM) Store at 2-8°C, protected from light

Required Materials Not Included:

- Phosphate Buffered Saline pH 7.4 (PBS) (no calcium/magnesium)

Detection/Imaging Guidelines:

- Ex/Em = 485/515 nm
- Fluorescence microscope filter set: FITC
- Flow cytometry channel: FITC

Live-Cell Imaging Assay Protocol:

1. Grow cells on vessel with coverslip bottom and treat them under desired experimental conditions.
   Note: Staurosporine treatment (5 µM) for 4 hours can be used as a positive control for most cell types.
2. Bring the NucView® 488 Caspase-3 Substrate to room temperature and add directly to cells to a final concentration of 5 µM.
   Ex: Add 1 µL to 200 µL medium per well in a 96-well plate.
   Note: Nuclear counterstaining such as DRAQ5 (Cat. No. 424101) can be added at the same time if desired.
3. Incubate cells at 37°C for 30 minutes or longer.
4. For endpoint analysis, wash cells gently with PBS before imaging.
   Note: Live cells can be imaged directly in the presence of Caspase-3 substrate if time point analysis is desired.
5. Image cells in FITC/GFP channel on a fluorescence microscope.
   Note: Cells stained with NucView® 488 Caspase-3 Substrate can undergo formaldehyde-

fixation, e.g. by adding Fixation Buffer (Cat. No. 420801). This probe is not compatible with alcohol-based fixatives. Detergent-based permeabilization may diminish signal intensity.

Flow Cytometry Assay Protocol:

1. Treat adherent or suspension cells under desired conditions.
   Note: Staurosporine treatment (5 µM) for 4 hours can be used as a positive control for most cell types
2. For adherent cells, detach cells and prepare cell suspension before proceeding.
3. Prepare cell suspension (about 10^6 cells/mL) in medium or buffer.
4. Bring the NucView® 488 Caspase-3 Substrate to room temperature and add directly to cells to a final concentration of 5 µM.
   Ex: Add 1 µL to 200 µL medium per well in a FACS tube. Co-stains, such as antibodies or live/dead stains can be added to cells at the same time.
5. Incubate cells at 37°C for 15-30 minutes or longer.
6. Optional: For endpoint analysis, or if samples cannot be analyzed immediately, cells can be washed with PBS and fixed for 10 min with 1 mL of Fixation Buffer (Cat. No. 420801). Wash cells with 2 mL PBS and resuspend in PBS or other buffer before analysis.
7. Analyze fluorescence intensity using the FITC (530/30 nm) channel.

Application References

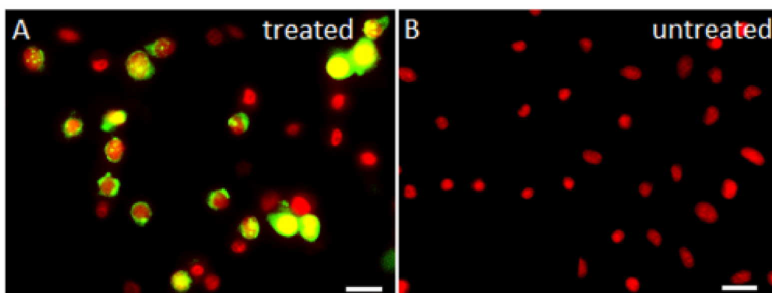
(PubMed link indicates BioLegend citation)

1. Omi J, *et al*. 2024. *JCB*. 223:2.
2. Kang D, *et al*. 2024. *Cell Death and Disease*. 15:26.
3. Choi DH, *et al*. 2023. *Front Onc*. 13:1252014.

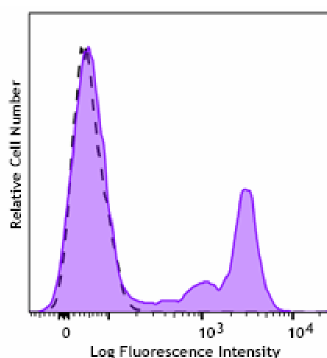
Antigen Details

Structure	Fluorogenic DNA dye coupled to the caspase DEVD recognition sequence
Function	Detection of caspase activity
Biology Area	Apoptosis/Tumor Suppressors/Cell Death, Cell Death
Gene ID	NA

Product Data



Live-cell fluorescence imaging of HeLa cells treated with 5 µM Staurosporine for 4 hours to induce caspase activity (panel A) or left untreated (panel B). Cells were then stained using NucView® 488 Caspase-3 Substrate and DRAQ5™ (nuclei) for 1 hour at 37°C. Images were captured in the FITC channel using a 40X objective. Scale bar: 10 µm



Jurkat cells were treated with Staurosporine for 4 hours and stained with NucView® 488 Caspase-3 Substrate (filled histogram). Open (dashed line) histogram represents untreated cells. Cells were analyzed on a flow cytometer.

For Research Use Only. Not for diagnostic or therapeutic use.

This product is supplied subject to the terms and conditions, including the limited license, located at www.biolegend.com/terms) ("Terms") and may be used only as provided in the Terms. Without limiting the foregoing, BioLegend products may not be used for any Commercial Purpose as defined in the Terms, resold in any form, used in manufacturing, or reverse engineered, sequenced, or otherwise studied or used to learn its design or composition without express written approval of BioLegend. Regardless of the information given in this document, user is solely responsible for determining any license requirements necessary for user's intended use and assumes

all risk and liability arising from use of the product. BioLegend is not responsible for patent infringement or any other risks or liabilities whatsoever resulting from the use of its products.

BioLegend, the BioLegend logo, and all other trademarks are property of BioLegend, Inc. or their respective owners, and all rights are reserved.

8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587