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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

NucView® 530 Caspase-3 Substrate

Catalog Numbers

10406-T	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO, 10 uL
10406	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO, 100 uL
10408-T	NucView® 530 Caspase-3 Substrate, 1 mM in PBS, 10 uL
10408	NucView® 530 Caspase-3 Substrate, 1 mM in PBS, 100 uL

Storage and Handling

Store 10406 NucView® 530 Caspase-3 Substrate, 1 mM in DMSO at 4ºC.
Store 10408 NucView® 530 Caspase-3 Substrate, 1 mM in PBS at -20ºC.

Protect from light. When stored as directed, product is stable for at least 6 months from the date it is received. **Centrifuge vial briefly to collect contents at bottom of vial before opening.**

Spectral Properties

Absorption/emission maxima of NucView® 530 dye with DNA: 528/563 nm (Fig. 1)

Product Description

NucView® 530 Caspase-3 Substrate, 1 mM in DMSO, provides a convenient tool for detecting apoptosis in intact cells based on caspase-3/7 activity using either confocal microscopy or flow cytometry.

In contrast to other fluorogenic caspase substrates or fluorescent caspase inhibitor based (FLICA) assays, NucView® caspase-3 substrates can be used to detect caspase-3/7 activity within individual intact cells without inhibiting apoptosis progression. NucView® substrates consist of a fluorogenic DNA dye coupled to the caspase-3/7 DEVD recognition sequence. The substrate, which is initially non-fluorescent, penetrates the plasma membrane and enters the cytoplasm. In apoptotic cells, caspase-3/7 cleaves the substrate, releasing the high-affinity DNA dye, which migrates to the cell nucleus and stains DNA with fluorescence. Thus, NucView® caspase-3 substrates are bifunctional, allowing detection of caspase-3/7 activity and visualization of morphological changes in the nucleus during apoptosis. The staining is also formaldehyde-fixable.

NucView® 530 Caspase-3 Substrate stains apoptotic cell nuclei with orange fluorescence, for detection in the Cy®3 channel by fluorescence microscopy or the PE channel by flow cytometry. NucView® 530 can be used for multi-color imaging with blue, green, or far-red fluorescent probes. Note that when excited by the 488 nm laser line, NucView® 530 also fluoresces in the FITC channel, and therefore cannot be analyzed together with green probes by flow cytometry.

NucView® 530 Caspase-3 Substrate is offered in DMSO and PBS (phosphate-buffered saline) formulations. The substrate in PBS is formulated for use in cells that are sensitive to DMSO toxicity. In non-DMSO sensitive cell types, adding DMSO during the substrate incubation may enhance NucView® 530 staining.

Biotium also offers blue fluorogenic NucView®405 Caspase-3 Substrate, and green fluorogenic NucView®488 Caspase-3 Substrate and kits (see Related Products).

References

Cen H, et al. FASEB J. 22, 2243–2252 (2008).

Assay protocols

Assay Optimization

Protocols for endpoint assays are provided below. NucView® substrates also can be incubated with cells continuously at 37°C for time course studies. Optimal substrate concentration for NucView® 530 may range between 1-10 uM, see protocols below for recommended starting concentrations. Cells can be incubated with substrate in culture medium, PBS, or other buffer of your choice. DMSO may increase cell permeability of the substrate, therefore the final concentration of DMSO should be controlled in samples that are compared directly. Media change or washing after substrate incubation is optional.

Controls

We recommend that you perform the following controls:

1. Negative control with cells not induced to undergo apoptosis
2. Positive control with cells induced to undergo apoptosis

For flow cytometry

1. Induce apoptosis by desired methods. Remember to include an untreated cell sample as a control.
2. For adherent cells, detach cells from culture substrate using trypsin or another cell dissociation method prior to performing the assay.
3. Resuspend cells at a density of 10^6 cells/mL in medium or buffer.
4. Pipette 0.2 mL cell suspension into a flow cytometry test tube.
5. Prepare a 0.2 mM NucView® 530 substrate solution by mixing 1 uL of 1 mM substrate stock solution with 4 uL medium or buffer. Add 2 uL of the 0.2 mM substrate solution to 200 uL cells and immediately mix for a final substrate concentration of 2 uM. Optimal substrate concentration may vary, see Assay Optimization.
6. Incubate cells at room temperature for 15-30 minutes, protected from light.
7. Add 300 uL medium or PBS to each tube and analyze by flow cytometry. Measure fluorescence in the PE detection channel (488 nm excitation, 575/26 nm emission). Note that when excited by the 488 nm laser line, NucView® 530 also fluoresces in the FITC channel, and is not recommended for multi-color flow cytometry analysis in combination with green fluorescent probes.

For microscopy

1. Induce apoptosis by desired methods. Remember to include an untreated cell sample as a control.
2. Prepare medium or PBS containing 2 uM NucView® 530 substrate by adding 1 uL of 1 mM substrate stock solution to 500 uL medium or buffer. Optimal substrate concentration may vary, see Assay Optimization.
3. Replace cell culture medium with medium or PBS containing 2 uM NucView® 530 substrate (prepared in step 2). Incubate cells with substrate at room temperature for 30 minutes or longer.

   Note: localized high background can result if concentrated substrate is added directly to buffer or medium in the culture well.
4. Image cells by confocal microscopy in the Cy®3 channel. Media change or washing is optional.

   Note: For confocal imaging, NucView® 530 should be imaged on a separate track from green fluorescent probes, to minimize bleed-through into the green channel. It is recommended to include single color controls in multi-color fluorescence imaging experiments to control for cross-talk between fluorescence channels.

Note: NucView® 530 staining is retained after formaldehyde fixation. Formaldehyde-fixed NucView® 530-stained cells can be permeabilized with 0.1% Triton X-100 for subsequent immunostaining; however, staining brightness may be reduced after permeabilization and washing.

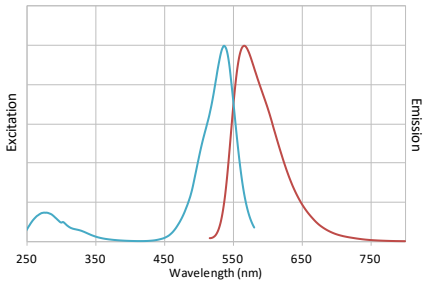

Figure 1. Excitation and emission spectra of NucView® 530 dye in the presence of dsDNA.

Please visit our website at **www.biotium.com** to view our full selection of products for cell viability and apoptosis detection,along with hundreds of other products for cell biology, genomics, and proteomics research.

Related Products

Catalog number	Product
10405	NucView®405 Caspase-3 Substrate, 1 mM in DMSO
10407	NucView®405 Caspase-3 Substrate, 1 mM in PBS
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10403	NucView® 488 Caspase-3 Substrate, 1 mM in PBS
10404	Ac-DEVD-CHO Caspase-3 Inhibitor
30029	NucView® 488 Caspase-3 Substrate Assay Kit for Live Cells
30067	Dual Apoptosis Assay Kit with NucView® 488 Caspase-3 Substrate & CF®594 Annexin V
30076	Dual Apoptosis Assay Kit with NucView® 488 Caspase-3 Substrate & CF®640R Annexin V
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit
30072	NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit
32002-32010	Live-or-Dye™ Fixable Viability Staining Kits
30065	Apoptosis & Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus
30060	CF®488A Annexin V and 7-AAD Apoptosis Kit
30061	CF®488A Annexin V and PI Apoptosis Kit
30001	JC-1 Mitochondrial Membrane Detection Kit
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
30074	CF®640R TUNEL Assay Apoptosis Detection Kit
80027	PathoGreen™ Histofluorescent Stain

Frequently Asked Questions

Question	Answer
How stable are NucView® Caspase-3 Substrates?	NucView® substrates are very stable when stored at 4°C, protected from light. DMSO stocks are stable to multiple freeze/thaw cycles at 4°C. Users have reported performing time course assays with NucView® 488 Caspase-3 Substrate for 4-5 days at 37°C.
When should I add NucView® Caspase-3 Substrate to my cells?	NucView® caspase-3 substrates can be added to the cells at the start of the experiment or at the end. NucView® Caspase-3 Substrates do not affect the time course of apoptosis progression, allowing caspase activity to be monitored in real time.
What instruments are compatible with NucView® Caspase-3 Substrates?	Blue fluorogenic NucView® 405 Caspase-3 Substrate is recommended for detection with 405 nm laser excitation by flow cytometry or confocal microscopy. Green fluorogenic NucView® 488 Caspase-3 Substrate is compatible with instruments that can excite and collect green fluorescence. Orange fluorogenic NucView® 530 caspase-3 substrate can be detected by fluorescence or confocal microcopy in the Cy®3 channel, or flow cytometry in the PE channel.
What cell types can be used with NucView® Caspase-3 Substrates?	NucView® 405 and NucView® 530 have been tested with Jurkat, HeLa, CHO, and MCF-7 cells. Green fluorogenic NucView® 488 Caspase-3 Substrate has been reported to work in a wide variety of primary cells and immortalized cell lines in the published scientific literature. Visit www.biotium.com to download a list of cell types and references.
Can NucView® Caspase-3 Substrates be used for tissue staining?	NucView® substrates have not been validated at Biotium for live tissue staining. There are publications reporting the use of green fluorogenic NucView® 488 caspase-3 substrate use in embryonic tissues and 3-dimensional cell culture. Visit www.biotium.com to download a list of NucView® publications. NucView® substrates cannot be used in fixed cells or tissues.
Can I fix NucView® staining for subsequent immunostaining?	Yes. We recommend fixation with 2-4% paraformaldehyde for 10-15 minutes at room temperature. Over-fixing can cause the signal to decrease. NucView® staining can withstand permeabilization with 0.1% Triton X-100, although signal intensity may be reduced after permeabilization and washing.
How long can I monitor NucView® staining under the microscope?	As with other fluorescence based probes, photobleaching may during imaging. How long you can view NucView® staining under the microscope depends on several factors including the initial signal strength and the intensity of the excitation source.
Why didn't Ac-DEVD-CHO inhibit NucView® staining in my cells?	Ac-DEVD-CHO is a reversible competitive inhibitor with limited cell permeability, and may not be sufficient to block high levels of caspase-3 activity. Adding an irreversible inhibitor like Z-DEVD-FMK before or after apoptosis induction may more effectively inhibit caspase activity.
How specific are NucView® Caspase-3 Substrates for caspase-3?	Like other caspase-3 substrates, NucView® Caspase-3 Substrates are based on a DEVD caspase-3 consensus sequence that also can be cleaved by caspase-7. Other caspases may also cleave DEVD substrates due to overlapping substrate specificity among caspases.
Do you offer NucView® substrates for other caspases or with different dye colors?	Biotium currently offer blue fluorogenic Nucview 405 Caspase-3 Substrate, green fluorogenic NucView® 488 Caspase-3 Substrate, and orange fluorogenic NucView® 530 Caspase-3 Substrate. Additional NucView® substrates are in development.