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Product Information

NucView™ 488 Caspase-3 Assay Kit for Live Cells

Catalog Number: 30029-T (25 assays), 30029 (100 assays)

Kit Contents

Component	30029-T 25 assays	30029 100 assays
NucView 488 Caspase-3 Substrate, 0.2 mM in DMSO	99925-T 125 uL	99925 2 X 250 uL
Caspase-3 inhibitor Ac-DEVD-CHO, 2 mM in DMSO	99901 20 uL	99969 100 uL

Number of fluorescence microscopy assays may vary depending on the size of culture vessel and staining volume used.

Storage and Handling

Store at 4ºC. When stored as directed, the kit is stable for at least 6 months from the date it is received.

Spectral Properties

Absorption/emission maxima of NucView 488 bound to DNA: 500/530 nm (Fig. 1)

Product Description

NucView 488 Caspase-3 Assay Kit for Live Cells contains NucView 488 Caspase-3 Substrate and caspase-3/7 inhibitor Ac-DEVD-CHO. This kit provides a convenient tool for profiling apoptotic cell population based on caspase-3/7 activity using either fluorescence microscopy or flow cytometry.

In contrast to other fluorogenic caspase substrates or fluorescent caspase inhibitor based (FLICA) assays, NucView 488 Caspase-3 Substrate can be used to detect caspase-3/7 activity within individual intact cells without inhibiting apoptosis progression. The substrate consists 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 bright green fluorescence. Thus, NucView 488 Caspase-3 Substrate is bifunctional, allowing detection caspase-3/7 activity and visualization of morphological changes in the nucleus during apoptosis. NucView 488 staining is formaldehyde-fixable and compatible with subsequent immunostaining.

References

Cen H, et al. FASEB J. 22, 2243–2252 (2008); Monaco, G., et al. Cell Death and Differentiation DOI 10.1038/cdd.2011.97 (2011); Schmitt, H., et al. Diabetologia DOI 10.1007/s00125-011-2133-5 (2011).

Please visit www.biotium.com to download a list of cell types tested with NucView 488 Caspase-3 Substrate with references.

Assay protocols

Assay Optimization:

Protocols for basic endpoint assays are provided below. NucView 488 substrate also can be incubated with cells for extended periods for time course studies (see Frequently Asked Questions, next page). Cell density, substrate concentration, and inhibitor concentration may require optimization. Optimal substrate concentration may vary between 1-10 uM. Cell can be incubated with substrate in culture medium, PBS, or other buffer of your choice. For adherent cells, we recommend removing medium and replacing with fresh medium containing substrate because high background can result in the area where concentrated substrate is added to the well. Media change or washing after incubation with substrate 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
3. Inhibitor control with cells induced to undergo apoptosis and incubated with caspase-3/7 inhibitor prior to addition of NucView 488 Caspase-3 Substrate

Ac-DEVD-CHO caspase-3 inhibitor controls:

The caspase-3/7 inhibitor Ac-DEVD-CHO included in the kit can be used to confirm the caspase-3/7 dependence of Nucview 488 fluorescence signal. For inhibitor control samples, the final concentration of inhibitor should be at least 2-fold higher than the final substrate concentration (for example, use 10 uM Ac-DEVD-CHO when using 5 uM NucView 488 substrate). Incubate samples with Ac-DEVD-CHO for 15-30 minutes at room temperature before adding substrate, and include inhibitor during incubation with the substrate. Ac-DEVD-CHO is a reversible competitive inhibitor. In some cell types, effective caspase-3/7 inhibition may require the use of an irreversible caspase-3/7 inhibitor such as Z-DEVD-FMK, or may require addition of inhibitor before or during apoptosis induction.

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 NucView 488 Caspase-3 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. For inhibitor control samples only, treat with Ac-DEVD-CHO at this step (see above).
6. Add 5 uL of 0.2 mM NucView 488 substrate stock solution to tube and mix well to obtain a final NucView 488 substrate concentration of 5 uM (see Assay Optimization).
7. Incubate cells at room temperature for 15-30 minutes, protected from light.
8. Add 300 uL medium or PBS to each tube and analyze by flow cytometry. Measure fluorescence in the green detection channel (excitation/emission: 485/515 nm).

For fluorescence microscopy

1. Induce apoptosis by desired methods. Remember to include an untreated cell sample as a control.
2. For inhibitor control samples only, treat with Ac-DEVD-CHO at this step (see above).
3. Replace medium with fresh medium or PBS containing 5 uM NucView 488 substrate stock solution (see Assay Optimization). For inhibitor controls, inhibitor should be included during incubation with substrate.
4. Incubate cells with substrate at room temperature for 30 minutes or longer.
5. Cells can observed directly in medium containing substrate. For endpoint analysis, wash cells with PBS and observe cells by fluorescence microscopy in PBS using filter sets for green fluorescence (excitation/emission: 485/515 nm).

For fluorescence microplate reader

1. Grow adherent cells in a black 96-well plate; for suspension cells, adjust density to 10^6 cells/mL and pipette 0.2 mL cell suspension into each well.
2. Induce apoptosis in cells by desired methods. Remember to include an untreated cell sample as a control. Note: cells may be treated in tubes or flasks and then aliquoted into plate wells for assay.
3. For inhibitor control samples only, treat with Ac-DEVD-CHO at this step (see above).
4. For suspension cells, add substrate directly to wells and mix well. For adherent cells, replace medium with fresh medium or PBS containing 5 uM NucView 488 substrate (see Assay Optimization). For Ac-DEVD-CHO inhibitor controls, inhibitor should be present during incubation with substrate.
5. Incubate cells at room temperature for 15-30 minutes, protected from light.
6. For suspension cells, gently shake plate to resuspend cells. Read fluorescence on a plate reader at settings close to 488 nm excitation and 520 nm emission cut-off. Bottom read is recommended for adherent cells. Inaccurate readings may result from variability in density of adherent cells.

Notes:

a. Cells can be counterstained with Hoechst 33342 dye (catalog number 40046) at a final concentration of 1 uM to stain all cell nuclei with blue fluorescence (excitation/emission: 346/460 nm)
b. NucView 488 staining is formaldehyde-fixable. NucView 488 staining is not compatible with methanol fixation.
c. Formaldehyde-fixed NucView 488-stained cells can be permeabilized with 0.1% Triton X-100 for subsequent immunostaining; however, staining brightness may be diminished after permeabilization and washing.

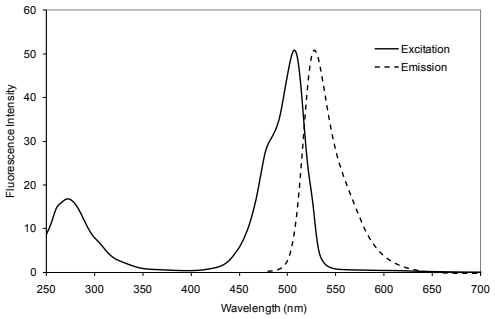

Figure 1. Excitation and emission spectra of enzymatically-cleaved NucView 488 Caspase-3 Substrate in the presence of dsDNA.

Related Products

Catalog number	Product
10402	NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in DMSO
10403	NucView™ 488 Caspase-3 Enzyme Substrate, 1 mM in PBS
10405	NucView™ 405 Caspase-3 Enzyme Substrate, 1 mM in DMSO
30067	Dual Apoptosis Assay Kit with NucView™ 488 Caspase-3 Substrate & CF™594-Annexin V
30062	NucView™ 488 and MitoView™ 633 Apoptosis Kit
30072	NucView™ 488 and RedDot™2 Apoptosis and Necrosis Kit
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
70055	MitoView™ 633
30063	CF™488A TUNEL Assay Apoptosis Detection Kit
30064	CF™594 TUNEL Assay Apoptosis Detection Kit

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.

Frequently Asked Questions

Question	Answer
How stable is NucView 488 Caspase-3 Substrate?	The substrate is very stable. Some 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 488 Caspase-3 Substrate to my cells?	NucView 488 Caspase-3 Substrate can be added to the cells at the start of the experiment or at the end. NucView 488 Caspase-3 Substrate does not affect the time course of apoptosis progression, so a major advantage of NucView 488 Caspase-3 Substrate compared to other apoptosis assays is that it can be used to monitor caspase-3 activity in real time.
What instruments are compatible with NucView 488 Caspase-3 Substrate?	NucView 488 Caspase-3 Substrate is compatible with instruments that can excite and collect green fluorescence.
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 488 Caspase-3 Substrate 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 488 staining can withstand permeabilization with 0.1% Triton X-100, although signal intensity may be diminished after permeabilization and washing. Methanol fixation is not recommended.
How long can I monitor NucView 488 Caspase-3 Substrate under the microscope?	As with other fluorescence based probes, photobleaching may during imaging. How long you can view NucView 488 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 very high levels of caspase-3 activity. In our experience, inhibitor treatment may reduce the overall fluorescence intensity of cell staining, but typically not to the level of untreated control cells. 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.
What cell types can be used with NucView 488 Caspase-3 Substrate?	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.
Do you offer NucView substrates for other caspases or with different dye colors?	Biotium currently offer blue fluorogenic Nucview 405 Caspase-3 Substrate and green fluorogenic NucView 488 Caspase-3 Substrate. Additional NucView substrates are in development.