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Product Information

Dual Apoptosis Assay Kit with NucView™488 Caspase-3 Substrate & Sulforhodamine 101-Annexin V (Texas Red®-Annexin V)

Catalog Number: 30030

Unit Size: 50 flow cytometry assays (200 uL assay volume).
Number of fluorescence microscopy staining assays may vary based on size of culture vessel and staining volume used.

Kit Contents

- NucView™488 Caspase-3 Substrate, 0.2 mM in DMSO, 250 uL
- Sulforhodamine 101-Annexin V (Texas Red®-Annexin V) 50 ug/mL in TE buffer, pH 7.5/0.1% BSA/0.1% NaN_3, 250 uL
- 5X Annexin V Binding Buffer, 15 mL
- Caspase-3 Inhibitor Ac-DEVD-CHO, 2 mM in DMSO, 100 uL

Storage and Handling

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

Product Description

NucView™488 and Sulforhodamine 101-Annexin V (Texas Red®-Annexin V) Dual Apoptosis Assay Kit allows simultaneous detection of caspase-3/7 activation and phosphatidylserine (PS) translocation in apoptotic cells by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

Traditional fluorogenic caspase substrates require cell lysis and cannot be used to measure caspase activity in live cells; furthermore such assays measure only the average caspase activity in a cell population. Fluorescently-labeled caspase inhibitor assay (FLICA) reagents can enter live cells to detect caspase activity, but because FLICA probes are also irreversible caspase inhibitors, they cannot be used to follow caspase activity in real time. In contrast, NucView™488 Caspase-3 substrate detects caspase-3 activity within individual intact cells without interfering with caspase-3 activity. NucView™488 Caspase-3 substrate is a novel fluorogenic substrate consisting of a DNA dye and a DEVD substrate moiety specific for caspase-3/7. The substrate, which is initially non-fluorescent, crosses the cell membrane to enter the cytoplasm, where it is cleaved by caspase-3/7 to release a high-affinity DNA dye, which migrates to the cell nucleus to stain the nucleus with bright green fluorescence, allowing caspase-3/7 detection and visualization of apoptotic nuclear morphology in adherent or suspension cells.

During apoptosis, phosphatidylserine (PS) is translocated from the inner to the outer surface of the cell, allowing the dying cell to be engulfed by phagocytic cells. Annexin V is a 35 kD Ca^{2+}-dependent phospholipid binding protein with a high affinity for PS. Fluorescent conjugates of annexin V can be used to label apoptotic cells by binding to PS exposed on the outer leaflet of the plasma membrane. The deep red fluorescence of Texas Red® (excitation/emission: 596/615 nm) is well separated from the green fluorescence of DNA-bound NucView™488.

References

Cen H, et al. FASEB J. 22, 2243–2252 (2008).
Martin, SJ, et al. J Exp Med. 182(5):1545-56 (1995).

Assay Protocols

Controls:
We recommend that you performing 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™ substrate

Assay Optimization:
The concentrations of NucView™488 substrate Ac-DEVD-CHO inhibitor provided in this protocol may require optimization depending on cell type. Optimal substrate concentration may vary between 1-10 uM. Use a final concentration of caspase-3 inhibitor Ac-DEVD-CHO that is at least 2-fold higher than the substrate concentration. Caspase-3 inhibitor Ac-DEVD-CHO is a reversible competitive inhibitor. In some cell types, 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 in cells by desired methods. Remember to include an untreated cell sample as a control.
2. Prepare 1X Binding Buffer by diluting 5X Annexin V Binding Buffer 1:5 with dH_2O.
3. Pellet cells and discard supernatant. For adherent cells, detach cells from culture plate with trypsin or other cell dissociation method prior to staining.
4. Resuspend cells in 1X Binding Buffer at a density of 10^6 cells/mL.
5. Pipette 0.2 mL cell suspension into a flow cytometry test tube.
6. For inhibitor control sample, add 1 uL caspase-3/7 inhibitor Ac-DEVD-CHO directly to the cell suspension and mix well to obtain a final inhibitor concentration of 10 uM (see Assay Optimization).
7. Incubate cells with inhibitor at room temperature for at least 15 minutes.
8. Add 5 uL of 0.2 mM NucView™ 488 Caspase-3 substrate stock solution and 5 uL Sulforhodamine 101-Annexin V (Texas Red®-Annexin V) stock solution directly into cell suspension and mix gently. This yields a final NucView™ 488 Caspase-3 substrate concentration of 5 uM (see Assay Optimization).
9. Incubate cells at room temperature, protected from light, for 15-30 minutes.
10. Add 400 uL 1X Binding Buffer to each tube and measure fluorescence in FITC (excitation/emission 485/515 nm) and Texas Red® (excitation/emission 594/615) channels.

For fluorescence microscopy

1. Induce apoptosis in cells by desired methods. Remember to include an untreated cell sample as a control.
2. Prepare 1X Binding Buffer by diluting 5X Annexin V Binding Buffer 1:5 with dH_2O.
3. Wash cells twice with PBS.
4. For inhibitor control samples, add 1X Binding Buffer containing 10 uM caspase-3 inhibitor Ac-DEVD-CHO. Prepare a sufficient volume of buffer to cover cells.
5. Incubate cell with inhibitor at room temperature for 15-30 min.
6. Prepare assay solution by adding 5 uL of 0.2 mM NucView™488 Caspase-3 substrate stock solution and 5 uL Sulforhodamine 101-Annexin V (Texas Red®-Annexin V) stock solution per 200 uL 1X Binding Buffer. This yields a final NucView™ 488 Caspase-3 substrate concentration of 5 uM. For inhibitor control samples, include 10 uM inhibitor (see Assay Optimization). Prepare sufficient volume of solution to completely cover cells.
7. Incubate cells with assay solution at room temperature, protected from light, for 30 minutes.
8. Wash cells twice with 1X Binding Buffer.
9. Add a sufficient volume of 1X Binding Buffer to completely cover cells.
10. Observe fluorescence using FITC and Texas-Red® filter sets.

Optional: cells can be counterstained with Hoechst dye (catalog number 40046, 1 uM final concentration) to visualize cell nuclei. Formaldehyde fixation may be performed for long term preservation of cell staining. Annexin V binding to PS is calcium-dependent, therefore buffers used for fixation should contain 1.25 mM calcium chloride ($CaCl_2$).

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Spectra

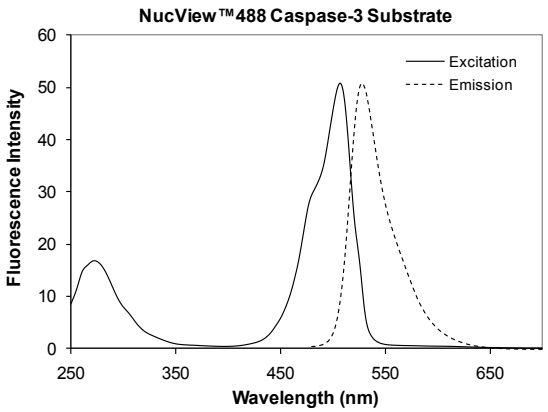

Figure 1. Excitation and emission spectra of enzymatically-cleaved NucView™488 Caspase-3 Substrate in the presence of dsDNA.

Related Products

Catalog number	Product
30067	Dual Apoptosis Assay Kit with NucView™ 488 caspase-3 substrate and CF™594-Annexin V
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
30029	NucView™ 488 Caspase-3 Assay Kit for live cells
30062	NucView™488 and MitoView™633 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

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
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 with NucView, 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.
Can I fix cells after performing the Dual Apoptosis Assay?	Yes. We recommend fixation with 2-4% paraformaldehyde for 10-15 minutes at room temperature. Over-fixing can cause the signal to decrease. To preserve Annexin V staining, include 1.25 mM $CaCl_2$ in all buffers. Methanol fixation is not recommended. NucView™ 488 staining can withstand permeabilization with 0.1% Triton X-100 for subsequent immunostaining, although signal intensity may be diminished after permeabilization and washing. Annexin V staining cannot withstand detergent permeabilization.
How specific is NucView™ 488 Caspase-3 Substrate 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.
Can the Dual Apoptosis Assay be used for tissue staining?	The Dual Apoptosis assay cannot be used in fixed cells or tissue sections. The assay has not been validated by Biotium for live tissue staining.