

Product Information

NucView® 488 and CF®640R Annexin V Dual Apoptosis Assay Kit for Live Cells

Catalog Number: 30073

Unit Size: 50 flow cytometry assays

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

Kit Contents

- NucView® 488 Caspase-3 Substrate, 0.2 mM in DMSO, 250 uL (component 99925)
- CF®640R-Annexin V in TE buffer, pH 7.5/0.1% BSA/0.1% NaN₃, 250 uL (component 99992)
- 5X Annexin V Binding Buffer, 15 mL (component 99902)
- Caspase-3 Inhibitor Ac-DEVD-CHO, 2 mM in DMSO, 100 uL (component 99969)

Storage and Handling

Store at 4°C, protected from light. When stored as recommended the kit is stable for at least 6 months from the date of receipt.

Product Description

NucView® 488 and CF®640R 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-3/7 activity within individual intact cells without interfering with caspase-3/7 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 Ca²⁺-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. CF®640R is an exceptionally bright and photostable far red dye with excitation/emission maxima at 642/662 nm, spectrally similar to Cy®5.

References

- Cen H, et al. DEVD-NucView488: A novel class of enzyme substrates for real-time detection of caspase-3 activity in live cells. *FASEB J.* 22, 2243–2252 (2008).
- Martin, SJ, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J Exp Med.* 182(5):1545-56 (1995).

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 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 dH₂O.
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 10⁶ cells/mL.
5. Pipette 200 uL 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 CF®640R 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 Cy®5 (excitation/emission 642/662 nm) 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 dH₂O.
3. Wash cells twice with PBS.
4. For inhibitor control samples, add 1X Binding Buffer containing 10 μ M 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 μ L of 0.2 mM NucView® 488 Caspase-3 substrate stock solution and 5 μ L CF@640R-Annexin V stock solution per 200 μ L 1X Binding Buffer. This yields a final NucView® 488 Caspase-3 substrate concentration of 5 μ M. For inhibitor control samples, include 10 μ M 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 Cy@5 filter sets.

Optional: cells can be counterstained with Hoechst dye (catalog number 40046, 1 μ M 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 (CaCl₂).

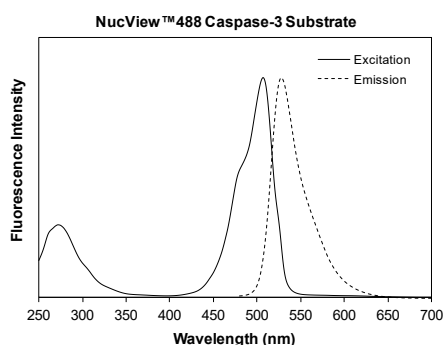


Figure 1. Excitation and emission spectra of enzymatically-cleaved NucView®488 caspase-3 substrate in the presence of dsDNA.

Related Products

Catalog number	Product
30067	Dual Apoptosis Assay with NucView®488 and CF@594 Annexin V
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10406	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO
30065	Apoptotic & Necrotic Cell Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cell Quantification 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
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 CF@ dye bioconjugates, including antibodies, antibody labeling kits, phalloidin, annexin V and α -bungarotoxin, as well as classic fluorescent nucleic acid dyes and hundreds of other products for life science research.

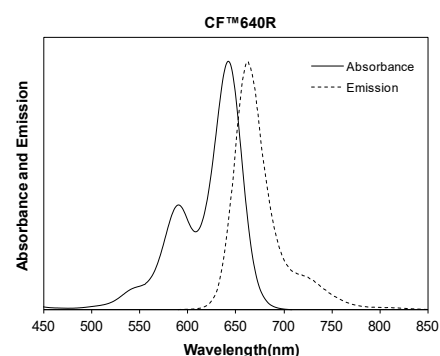


Figure 2. Absorbance and emission spectra of CF@640R conjugates.

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 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.
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.

NucView enzyme substrate technology is covered by granted US patents. CF dye technologies are covered by granted and pending US and international patents. Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use. Cy Dye is a registered trademark of GE Healthcare.