

# Product Information

## NucView® 488 Caspase-3 Substrate

### Catalog Numbers:

10402-T NucView® 488 Caspase-3 Substrate, 1 mM in DMSO, 10 µL  
10402 NucView® 488 Caspase-3 Substrate, 1 mM in DMSO, 100 µL  
10403-T NucView® 488 Caspase-3 Substrate, 1 mM in PBS, 10 µL  
10403 NucView® 488 Caspase-3 Substrate, 1 mM in PBS, 100 µL

### Storage and Handling

Store 10402 NucView® 488 Caspase-3 Substrate, 1 mM in DMSO at 4 °C.  
Store 10403 NucView® 488 Caspase-3 Substrate, 1 mM in PBS at -20 °C.

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

Abs/Ems maxima of NucView® 488 bound to DNA: 500/530 nm (Fig. 1)

### Product Description

NucView® 488 Caspase-3 Substrate provides a convenient tool for profiling apoptotic cell populations 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 of 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.

NucView® 488 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.

### 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](http://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 and substrate concentration may require optimization. Optimal substrate concentration may vary between 1–10 µM. Cells 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.

When using NucView® 488 Caspase-3 Substrate, 1 mM in PBS in non-DMSO sensitive cell types, adding DMSO during the substrate incubation may enhance NucView® 488 staining.

### 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 NucView® 488 caspase-3 assay.
3. Resuspend cells at a density of 10<sup>6</sup> cells/mL in medium or buffer.
4. Pipette 0.2 mL cell suspension into a flow cytometry test tube.
5. Add 1 µL of 1 mM substrate solution to 200 µL cells and immediately mix for a final substrate concentration of 5 µM. Optimal substrate concentration may vary, see Assay Optimization.
6. Incubate cells at room temperature for 15–30 minutes, protected from light.
7. Add 300 µL 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. Replace medium with fresh medium or PBS containing 5 µM NucView® 488 substrate stock solution (see Assay Optimization).
3. Incubate cells with substrate at room temperature for 30 minutes or longer.
4. Cells can be 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

Note: microplate readers designed to read fluorescence in solution generally have low sensitivity for localized intracellular stains like NucView®.

1. Grow adherent cells in a black 96-well plate; for suspension cells, adjust density to 10<sup>6</sup> 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 suspension cells, add substrate directly to wells and mix well. For adherent cells, replace medium with fresh medium or PBS containing 5 µM NucView® 488 substrate (see Assay Optimization).
4. Incubate cells at room temperature for 15–30 minutes, protected from light.
5. 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 µM 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. Fixed cells can be permeabilized with 0.1% Triton™ X-100, however, staining brightness may be diminished after permeabilization and washing.

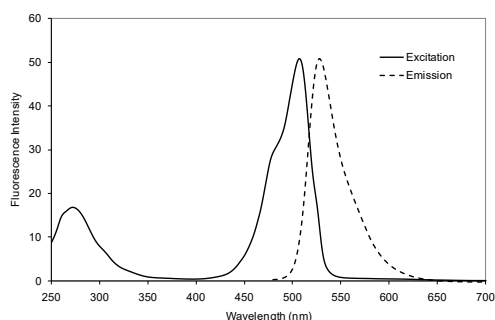


Figure 1. Excitation and emission spectra of enzymatically-cleaved NucView® 488 Caspase-3 Substrate in the presence of dsDNA.

Please visit our website at [www.biotium.com](http://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 Enzyme Substrate, 1 mM in DMSO
10407	NucView® 405 Caspase-3 Enzyme Substrate, 1 mM in PBS
10406	NucView® 530 Caspase-3 Enzyme Substrate, 1 mM in DMSO
10408	NucView® 530 Caspase-3 Enzyme Substrate, 1 mM in PBS
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
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
70055	MitoView™ 633
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit

## 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 <a href="http://www.biotium.com">www.biotium.com</a> 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 occur 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 <a href="http://www.biotium.com">www.biotium.com</a> 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, green fluorogenic NucView® 488 Caspase-3 Substrate, and orange fluorogenic NucView® 530 Caspase-3 Substrate. Additional NucView® substrates are in development.

NucView enzyme substrate technology is covered by U.S. patents.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

Triton is a trademark of The Dow Chemical Company.

**NucView® 488 Caspase-3 Substrate**

PSF006