

NucView™ 488 Caspase-3 Substrate  
Frequently Asked Questions

<b>Problem or Question</b>	<b>Answer or Suggestion</b>
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.
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. 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.
Is NucView™ 488 Caspase-3 Substrate compatible with flow cytometry or microplate readers?	Yes. NucView™ 488 Caspase-3 Substrate is compatible with instruments that can excite and collect green emission.
Can I fix NucView™ 488 Caspase-3 Substrate for subsequent immunostaining?	Yes. We recommend a gentle fix in 2-4% paraformaldehyde for 10-15 minutes. Over-fixing can cause the signal to decrease. NucView™ 488 staining can withstand permeabilization with 0.1% Triton X-100. Methanol fixation is not recommended.
Can NucView™ 488 Caspase-3 Substrate be used for tissue staining?	NucView™ 488 Caspase-3 Substrate has not been validated by Biotium for live tissue staining. However, we have had feedback from a customer who successfully used NucView™ 488 Caspase-3 Substrate for live zebrafish embryo staining. NucView™ 488 Caspase-3 Substrate cannot be used in fixed cells or tissues.
How long can I monitor NucView™ 488 Caspase-3 Substrate under the microscope?	As with all fluorescence based probes, bleaching will occur over time. 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.
The inhibitor DEVD-CHO does not seem to inhibit NucView™ 488 staining.	DEVD-CHO is a reversible inhibitor and may not sufficiently block caspase-3 activity. Adding an irreversible inhibitor like DEVD-FMK at the beginning of the experiment (before or at the time of apoptosis induction) may more effectively inhibit caspase activity.
How specific is NucView™ 488 Caspase-3 Substrate for caspase-3?	Like other caspase-3 substrates, NucView™ 488 Caspase-3 Substrate is based on a DEVD sequence that also can be cleaved by caspase-7.
Do you have NucView™ substrates for other caspases or with different fluorescent dye colors?	Additional NucView™ caspase substrates currently are in development.