BioTracker™ NucView® 530 Red Caspase-3 Dye (PBS)

Live Cell Dye

Cat. # SCT105

pack size: 100µL

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

Caspases are a family of protease enzymes that play essential roles in programmed cell death, termed apoptosis. Caspase-3 is a caspase protein that interacts with caspase-8 and caspase-9 and sequential activation of plays a central role in the execution-phase of cellular apoptosis.

The BioTracker™ NucView® 530 Red Caspase-3 Dye provides a convenient live cell 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, the BioTracker™ NucView® 530 Red Caspase-3 Dye 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 red fluorescence. Thus, NucView® 530 Caspase-3 Substrate is bifunctional, allowing detection of caspase-3/7 activity and visualization of morphological changes in the nucleus during apoptosis. The dye is formaldehyde-fixable and compatible with subsequent immunostaining. The dye 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.

NucView® 530 Caspase-3 Substrate stains apoptotic cell nuclei with orange fluorescence, for detection in the Cy®3 channel by fluorescence microscopy or the PE channel by flow cytometry. Note that when excited by the 488nm laser line, NucView® 530 also fluoresces in the FITC channel, and therefore cannot be analyzed together with green probes by flow cytometry.

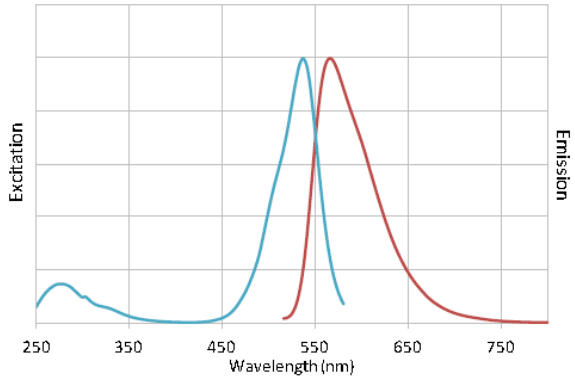

Figure 1. Excitation and emission spectra of enzymatically-cleaved BioTracker™ NucView® 530 Red Caspase-3 Dye in the presence of dsDNA.

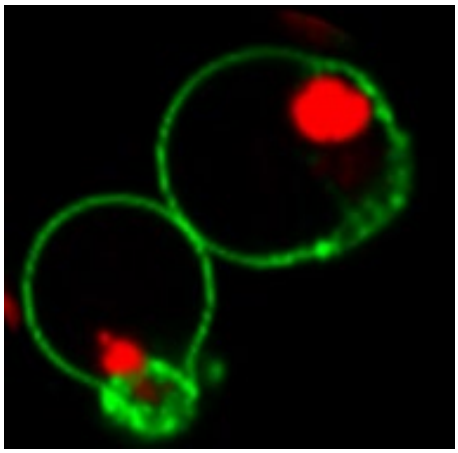

Figure 2. Apoptotic Jurkat cells stained with BioTracker™ NucView® 530 Red Caspase-3 Dye and CF™488 Annexin V (Green).

Storage

Store BioTracker™ NucView® 530 Red Caspase-3 Dye, 1mM in PBS at -20ºC. Protect From Light.

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.

Spectral Properties

Absorbance: 528nm
Emission: 563nm

Rev.1.0/2018-01-29/SCT105/NJA

Assay Protocol

Cell density and dye concentration may require optimization. Optimal dye concentration may vary between 1-10 uM. 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 dye is optional.

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® 530 caspase-3 assay.
3. Resuspend cells at a density of 10^6 cells/mL in medium or buffer.
4. Pipette 0.2mL cell suspension into a flow cytometry test tube.
5. Add 1µL of 1mM substrate solution to 200µL cells and immediately mix for a final substrate concentration of 5uM. Optimal substrate concentration may vary.
6. Incubate cells at room temperature for 15-30 minutes, protected from light.
7. Add 300µL medium or PBS to each tube and analyze by flow cytometry. Measure fluorescence in the red detection channel (excitation/emission: 528/563nm).

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 5µM NucView® 530 substrate stock solution.
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 red fluorescence (excitation/emission: 528/563nm).

Fluorescent Plate Reader

1. Grow adherent cells in a black 96-well plate; for suspension cells, adjust density to 10^6 cells/mL and pipette 0.2mL 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 5µM NucView® 530 substrate.
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 523nm excitation and 563nm 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 at a final concentration of 1µM to stain all cell nuclei with blue fluorescence (excitation/emission: 346/460 nm).

b. NucView® 530 staining is formaldehyde-fixable. NucView® 530 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.

Frequently Asked Questions

1. **Q. When do I add the dye to my cells? How stable is the dye? How long can I monitor the dye under the microscope?**
   A. The dye can be added anytime during the experiment. It does not affect apoptosis progression. The dye is very stable and can be view for up to 4-5 days in real-time experiments.

2. **Q. Can the dye be used on tissues or fixed cells? What cell types have been validated with the dye?**
   A. Yes. We recommend fixation with 2-4% paraformaldehyde for 10-15 minutes at room temperature. Over-fixing can cause the signal to decrease. The dye can withstand permeabilization with 0.1% Triton X-100, although signal intensity may be diminished after permeabilization and washing. Methanol fixation is not recommended. The dye works in a wide variety of primary cells and immortalized cell lines in the published scientific literature.