Product Information

NucSpot® Far-Red, 1000X in DMSO

Catalog Number/Unit Size
40085-T: 50 uL
40085: 0.5 mL

Storage and Handling
Store at -20°C, protected from light. Product is stable for at least 1 year from date of receipt when stored as recommended.

Spectral Properties
Excitation/Emission: 597/667 nm (with DNA) (Figure 1)

Product Description
NucSpot® Far-Red is designed to be an improvement over the popular flow cytometry dead cell dye 7-AAD. Like 7-AAD, NucSpot® Far-Red can be excited with the 488 nm laser for detection in the PE-Cy®5 channel. Due to its red-shifted emission compared to 7-AAD, it has less bleed-through fluorescence in the PE-Texas Red® channel, making it useful for flow cytometry multiplexing. NucSpot® Far-Red also is much brighter than 7-AAD in the APC channel with 633 nm excitation (Figure 2).

NucSpot® Far-Red is excluded by live cells, but stains necrotic and late apoptotic cells with compromised membrane integrity. NucSpot® Far-Red has negligible fluorescence until it binds to DNA, allowing it to be used in no-wash, homogenous assays. The dye also can be used for cell cycle analysis by flow cytometry in fixed and permeabilized cells. Unlike propidium iodide, RNase-treatment is not required for DNA content profiling with NucSpot® Far-Red.

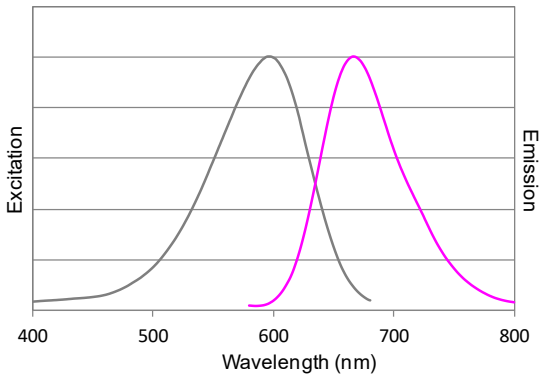

Figure 1. Normalized excitation and emission spectra of NucSpot® Far-Red in TE buffer with dsDNA.

Assay Protocols

NucSpot® Far-Red can be directly substituted for 7-AAD in any standard protocol. We recommend using it at 1:1000 dilution for live/dead discrimination or 1:100 dilution for cell cycle profiling. Example protocols are provided below.

For live/dead discrimination by flow cytometry

1. Adjust cells to 10^6 cells per mL in complete culture medium or buffer of your choice and aliquot 1 mL per flow tube.

   Note: cells can be stained anywhere between $5x10^5$ cells/mL to 10^7 cells per mL in 100 uL to 1 mL.
2. Add 1 uL of NucSpot® Far-Red 1000X Solution per mL of cells and mix.
3. Incubate 15 minutes at room temperature, protected from light.
4. Analyze by flow cytometry in the PE-Cy®5 channel or APC channel without washing the cells.

   Note: Cells can be fixed with formaldehyde and permeabilized with detergent after staining with NucSpot® Far-Red. The dye has better tolerance for fixation compared to other dead cell stains such as propidium iodide. However, dead cell fluorescence decreases about 3-fold and live cell fluorescence increases about 3-fold after fixation. We recommend performing flow cytometry analysis within 24 hours of fixation. For a stable fixable dead cell nuclear stain, see our Live-or-Dye NucFix™ Red (see Related Products).

For cell cycle profiling by flow cytometry analysis of DNA content

Materials required but not provided (see Related Products)

- Flow Cytometry Fixation/Permeabilization Kit (catalog no. 23006)
- 1X Phosphate buffered saline (PBS) (catalog no. 22020) or your preferred FACS buffer

Staining Protocol

1. Adjust cells to 10^7 cells per mL and aliquot 100 uL per flow tube.
2. Fix and permeabilize cells according the protocol for the Flow Cytometry Fixation/Permeabilization Kit, or use your preferred method.
3. Pellet the cells by centrifugation and wash with 1X PBS or FACS buffer.
4. Pellet the cells by centrifugation and resuspend in 100 uL buffer.
5. Add 1 uL NucSpot® Far-Red per tube and mix by gentle vortexing.
6. Incubate 15 minutes at room temperature, protected from light.
7. Add 400 uL PBS or FACS buffer per tube. Analyze by flow cytometry in the PE-Cy®5 channel or APC channel. Use a linear scale for fluorescence detection, and acquire data with a slow flow rate (~12 uL/minute).

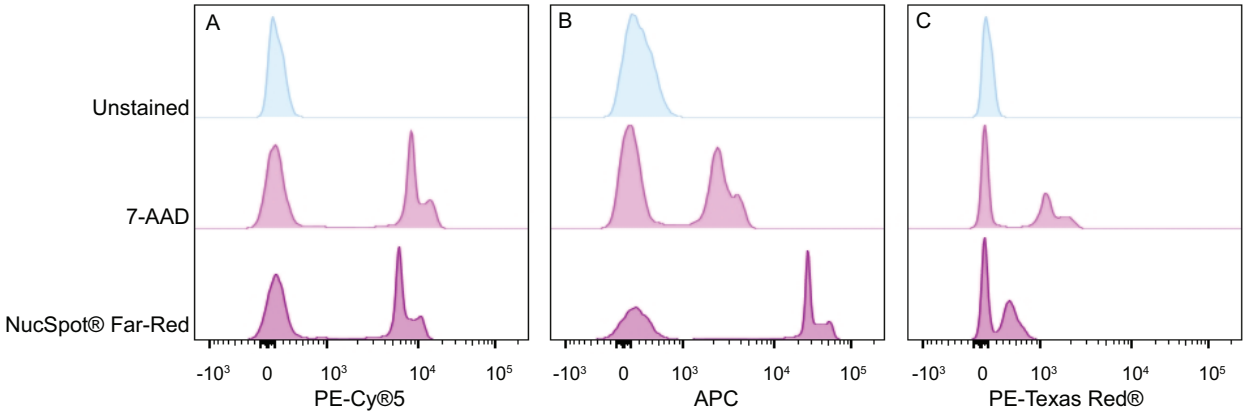

Figure 2. Comparison of live/dead staining with 7-AAD or NucSpot® Far-Red by flow cytometry analysis. A mixture of live and heat-killed Jurkat cells were left unstained (top), stained with 1 ug/mL 7-AAD (middle) or 1X NucSpot® Far-Red (bottom) and analyzed on a BD LSRII flow cytometer. The live cell peak is on the left, with low fluorescence similar to unstained cells, while the fluorescent dead cell peaks are shifted to the right. NucSpot® Far-Red has roughly comparable brightness to 7-AAD with 488 nm excitation in the PE-Cy®5 channel (488 nm laser, 660/20 BP filter) (A). The dye also is much brighter than 7-AAD with 633 nm excitation in the APC channel (633 nm laser, 660/20 BP filter) (B), with less spill-over in the PE-Texas Red® channel (488 nm laser, 610/20 BP filter) (C).

Related Products

Catalog number	Product
23006	Flow Cytometry Fixation/Permeabilization Kit
22023	Paraformaldehyde, 4% in PBS Ready-to-Use Fixative
22020	10X Phosphate Buffered Saline (PBS) (4L Cubitainer®)
22003	Mini Cell Scrapers
32010	Live-or-Dye NucFix™ Red
30068	ViaFluor® 405 SE Cell Proliferation Assay Kit
30086	ViaFluor® 488 SE Cell Proliferation Assay Kit
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
40048	Propidium Iodide Buffer, 50 ug/mL
40084	7-AAD Solution, 1 mg/mL
30072	NucView® 488 and RedDot™2 Apoptosis/Necrosis Kit
30060	CF®488A Annexin V and 7-AAD Apoptosis Kit
30061	CF®488A Annexin V and PI Apoptosis Kit
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit
30067	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®594 Annexin V
30073	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®640R Annexin V
40060	RedDot™1 Far-Red Nuclear Stain for live cells
40061	RedDot™2 Far-Red Nuclear Stain for dead or fixed cells
40083	NucSpot® 470 green nuclear stain for dead or fixed cells
40046	Hoechst 33342, 10 mg/mL in H_2O

Please visit our website at www.biotium.com for information on our life science research products, fluorescent CF® dye primary and secondary antibody conjugates, NucView® 488 real-time caspase-3 substrate, Live-or-Dye™ fixable viability stains, and many more fluorescent probes and kits for cell viability and apoptosis research.