

Product Information

NucSpot® 470, 1000X in DMSO

Catalog Number: 40083, 40083-T

Unit Size:
40083-T: 20 uL
40083: 200 uL

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Absorption/Emission: 460/546 nm (when bound to DNA) (Figure 1).

Product Description

NucSpot® 470 is a cell membrane-impermeant green fluorescent DNA stain. It is virtually non-fluorescent in the absence of DNA but fluoresces bright green upon DNA binding. While other green nucleic acid stains like TOTO®, TO-PRO®, or SYTOX® dyes stain both the nucleus and cytoplasm, NucSpot® 470 specifically stains the nucleus of fixed and permeabilized cells, as well as the nuclei of dead or permeabilized yeast. In live cultures, NucSpot® 470 is useful for selective staining of dead cells. NucSpot® 470 may be used for DNA content analysis of cell cycle by flow cytometry in fixed and permeabilized cells without requiring RNase treatment, unlike propidium iodide.

NucSpot® 470 has green fluorescence that can be imaged using standard settings for FITC. With an excitation maximum at 460 nm, it is an excellent match for instruments with blue LED excitation sources as well.

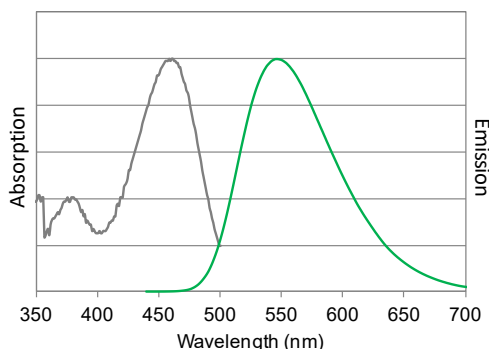


Figure 1. Normalized absorbance and emission of NucSpot® 470 when bound to DNA.

Experimental Protocols

Nuclear staining of fixed and permeabilized cells

1. Fix and permeabilize cells according to your standard protocol. NucSpot® 470 has been validated in formaldehyde-fixed/detergent permeabilized cells and methanol-fixed cells with no additional permeabilization step.
2. Dilute NucSpot® 470, 1000X in DMSO to a final concentration of 1X in PBS or other buffer. For example, add 1 uL of NucSpot® 470 to 1 mL of buffer.
Note: Optimal staining concentration may vary for different cell types or applications.
3. Incubate sample with diluted NucSpot® 470 for 10 minutes or longer at room temperature, protected from light. NucSpot® 470 can be included with secondary antibodies or other probes according to your preferred staining protocol.
4. Optional: Stained cells can be mounted in antifade mounting medium before imaging.
5. Image cells in the FITC channel. Washing is not necessary before imaging.

Selective staining of dead cells

1. Dilute NucSpot® 470 to a final concentration of 1X in complete cell culture medium or buffer of your choice.
2. Incubate cells with diluted NucSpot® 470 for 15 minutes or longer at room temperature or 37°C, protected from light. Live cells can be incubated with NucSpot® 470 for up to 4 hours at 37°C without obvious toxicity or staining of cells.
Note: After 4 hours, NucSpot® 470 staining is not stable and should not be used for long-term staining in live cultures.
3. Analyze cells by microscopy or flow cytometry in the FITC channel.

Cell cycle profiling by flow cytometry analysis of DNA content

1. Adjust cells to 10⁷ 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 (Cat. no. 23006), or use your preferred method.
Note: The Flow Cytometry Fixation/Permeabilization Kit (Cat. no. 23006) is recommended but not provided (see Related Products).
3. Pellet the cells by centrifugation and wash with 1X PBS or FACS buffer.
4. Pellet the cells by centrifugation and resuspend in 125 uL buffer.
Note: If using Biotium's Permeabilization Buffer (Cat. No. 22016 or kit 23006) or 0.1% Triton® X-100, NucSpot® 470 can be included in the permeabilization buffer for simultaneous permeabilization and staining. If a different fixation and permeabilization method is used, we recommend staining in buffer after fixation and permeabilization is complete.
5. Add 1 uL NucSpot® 470 (8X concentration, or 1:125 dilution) per tube and mix by gentle vortexing.
Note: When cell cycle profiling, a higher concentration of NucSpot® 470 is necessary to ensure a stoichiometric amount of dye is bound to the DNA.
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 FITC channel. Use a linear scale for fluorescence detection, and acquire data with a slow flow rate (~12 uL/minute).

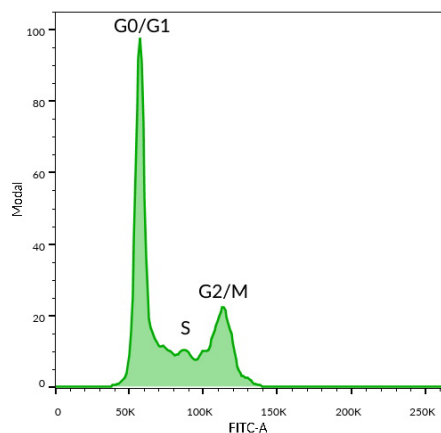


Figure 2. Cell cycle profiling by flow cytometric DNA content analysis in fixed/permeabilized cells using NucSpot® 470. Jurkat cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature, then stained with 8X NucSpot® 470 in PBS with 0.1% Triton® X-100 for 15 minutes at room temperature. Cells were analyzed on a BD® LSR II flow cytometer in the FITC channel on linear scale.

Related Products

Cat. No.	Product
40085	NucSpot® Far-Red, 1000X in DMSO
40081	NucSpot® Live 488 Nuclear Stain
40082	NucSpot® Live 650 Nuclear Stain
40060	RedDot™ 1 Far-Red Nuclear Stain, 200X in Water
40061	RedDot™ 2 Far-Red Nuclear Stain, 200X in DMSO
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23016	EverBrite™ Hardset with NucSpot® 640
23017	EverBrite TrueBlack® Hardset Mounting Medium
23018	EverBrite TrueBlack® Hardset with DAPI
23019	EverBrite TrueBlack® Hardset with NucSpot® 640
70058... 70086	LysoView™ Dyes
70070	MitoView™ 405
70054	MitoView™ Green
70055	MitoView™ 633
70075	MitoView™ 650
70068	MitoView™ 720
30050... 30086	ViaFluor® SE Cell Proliferation Kits
70062	ViaFluor® 488 Live Cell Microtubule Stain
70063	ViaFluor® 647 Live Cell Microtubule Stain
40050	Ethidium Homodimer III (EthD-III)
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
23006	Flow Cytometry Fixation/Permeabilization Kit
22015	Fixation Buffer
22016	Permeabilization Buffer

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates, cellular stains, and many more fluorescent probes and kits for cellular and molecular biology research.

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