Revised: August 21, 2023

Product Information

NucSpot® Nuclear Stains

Product List

Cat. No.	Unit Size	Product	Ex/Em (nm) (with DNA)
40083-T	20 uL	NucSpot® 470, 1000X in DMSO	461/547
40083	200 uL		
41040-T	20 uL	NucSpot® 500/515, 1000X in DMSO	497/513
41040	100 uL		
41033-T	20 uL	NucSpot® 555/570, 1000X in DMSO	559/566
41033	100 uL		
41036-T	20 uL	NucSpot® 568/580, 1000X in DMSO	572/583
41036	100 uL		
41037-T	20 uL	NucSpot® 594/615, 1000X in DMSO	603/613
41037	100 uL		
41034-T	20 uL	NucSpot® 650/665, 1000X in DMSO	653/671
41034	100 uL		
40085-T	50 uL	NucSpot® Far-Red, 1000X in DMSO	597/667
40085	500 uL		
41035-T	20 uL	NucSpot® 680/700, 1000X in DMSO	683/707
41035	100 uL		
41038-T	20 uL	NucSpot® 750/780, 1000X in DMSO	757/780
41038	100 uL		

Storage and Handling

Store NucSpot® Far-Red, 1000X in DMSO (Cat. No. 40085) at -20°C, protected from light. Store all other NucSpot® Nuclear Stains at 4°C, protected from light. Products are stable for at least 12 months from date of receipt when stored as recommended.

Product Description

NucSpot® Nuclear Stains are cell membrane-impermeant fluorescent DNA stains available in a variety of colors from green to near-infrared (near-IR). The stains have minimal fluorescence until they bind to DNA and can be used for no-wash nuclear staining. Unlike other nucleic acid dyes such as propidium iodide (PI), TOTO®, TO-PRO®, and similar dyes that stain both the nucleus and cytoplasm, NucSpot® Nuclear Stains selectively stain the nucleus in fixed and permeabilized cells without the need for RNase treatment.

NucSpot® Nuclear Stains also can be used for selective staining of dead cells in unfixed cell cultures for analysis by flow cytometry or fluorescence imaging. Several of the dyes can be continuously incubated with cells for multi-day imaging, see Table 1.

NucSpot® 470 and NucSpot® Far-Red can 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 around 460 nm, it is an excellent match for instruments with blue LED excitation sources as well. It can be used for DNA content analysis by flow cytometry. NucSpot® 470 can also be used for nuclear staining of dead or permeabilized yeast.
- NucSpot® 500/515 is a green fluorescent nuclear stain for the FITC channel. In addition to bright nuclear counterstaining of fixed cells, it can also be used for multi-day live/dead staining of mammalian cells in culture.
- NucSpot® 555/570 and NucSpot® 568/580 have orange and visible red fluorescence, respectively, and are nuclear-specific alternatives to PI and similar dyes.
- NucSpot® 594/615 has deep red fluorescence for the Texas Red® channel.
- NucSpot® 650/665 has far-red fluorescence with superior nuclear specificity compared to first-generation far-red nuclear stains such as RedDot™2 and Draq7™.
- NucSpot® Far-Red is a flow cytometry stain developed as an improved alternative to 7-AAD. It shows less bleed-through fluorescence in the PE-Texas Red® channel compared to 7-AAD and is ideal for selective detection of dead cells or DNA content analysis by flow cytometry without RNase treatment.
- NucSpot® 680/700 and NucSpot® 750/780 are spectrally unique DNA stains for far-red and near-IR detection.

Table 1. NucSpot® Nuclear Stains Detection Channels and Validated Applications

Cat. No.	NucSpot® Stain	Detection Channel	Validated Applications			
			Nuclear counterstaining	Live/dead discrimination	Multi-day live cell imaging	Flow cytometry cell cycle profiling
40083	NucSpot® 470	FITC	Yes	Yes	No	Yes
41040	NucSpot® 500/515	FITC*	Yes	Yes	Yes	No
41033	NucSpot® 555/570	Cy®3 or PE*	Yes	Yes	No	No
41036	NucSpot® 568/580	Cy®3 or PE*	Yes	Yes	Yes	No
41037	NucSpot® 594/615	Texas Red® or PE-Texas Red®*	Yes	Yes	Yes	No
41034	NucSpot® 650/665	Cy®5 or APC*	Yes	Yes	No	No
40085	NucSpot® Far-Red	PE-Cy®5 or APC †	No†	Yes (flow cytometry)†	No	Yes
41035	NucSpot® 680/700	Cy®5.5*	Yes	Yes	No	No
41038	NucSpot® 750/780	Cy®7 or APC-Cy®7*	Yes	Yes	Yes	No

* May show crosstalk in lower wavelength detection channels. Perform single-stain controls before combining with other probes.
† NucSpot® Far-Red is designed for flow cytometry optical systems and is not recommended for fluorescence microscopy.

Considerations for Staining Fixed Cells

- Some NucSpot® Stains may show crosstalk in lower wavelength detection channels, see Table 1 for details. Always perform single stain controls and optimize the staining concentration to reduce fluorescence crosstalk before combining NucSpot® stain with other fluorescent probes.
- NucSpot® Stains are cell membrane impermeant; cells must be fixed and permeabilized for nuclear counterstaining. The stains have been tested in formaldehyde-fixed cells permeabilized with 0.1% Triton® X-100, as well as methanol-fixed cells.
- Optimal staining concentration may vary for different cell types or applications. Concentrations lower than 1X may be optimal to prevent crosstalk between channels in multicolor experiments.
- NucSpot® Stains can be included with secondary antibodies or other probes according to your preferred staining protocol.
- Staining may appear around the nuclear rim first, then in the center of the nucleus. Longer incubation times (30 minutes or longer) or including 0.1% Triton® X-100 in the staining buffer can produce faster, more uniform nuclear staining.
- Staining intensity will continue to increase with longer incubation times.
- NucSpot® Stains have minimal fluorescence in the absence of DNA. Washing before detection is optional. Staining will be retained after washing.
- Samples may be mounted in antifade mounting medium before imaging. NucSpot® 555/565, NucSpot® 650/665, and NucSpot® 750/780 may not be compatible with antifade mounting medium containing phenylenediamine, such as VECTASHIELD® Mounting Medium.
- A higher stain concentration is used for cell cycle profiling of DNA content compared to other applications to ensure stoichiometric binding of the stain to the DNA. Not all NucSpot® Stains are suitable for DNA content analysis by flow cytometry, see Table 1 for validated applications.

Considerations for Live/Dead Discrimination

- NucSpot® Stains are cell membrane impermeant and, therefore, can be used for selective staining of dead cells in live cultures.
- Cells cannot be fixed prior to live/dead staining with NucSpot® Stains. We also do not recommend fixing cells after live/dead staining with NucSpot® Stains because dead-cell specificity will be lost after fixation.
- Table 1 lists NucSpot® Stains validated for multi-day live/dead staining with continuous incubation in live HeLa or MCF-7 cells for up to 72 hours.
- A lower stain concentration may be optimal for live dead discrimination for flow cytometry, we recommend titrating the stains between 1X and 0.05X.
- For multi-day incubation, we recommend using the lowest stain concentration that shows good live/dead discrimination and assessing for toxicity of the stain in your specific cell type.
- NucSpot® 555/565, NucSpot® 650/665, and NucSpot® 680/700 are not recommended for multi-day incubation. While these stains are stable in culture medium for up to 72 hours, live cell staining may increase with prolonged incubation times.
- NucSpot® 470 is not recommended for long-term staining (>4 hours) in live cultures due to unstable signal.
- NucSpot® Far-Red is recommended for flow cytometry, not microscopy. The dye has also not been evaluated for multi-day incubation in live cultures.

Experimental Protocols

Protocol for nuclear staining of fixed and permeabilized cells

Note: See Considerations for Staining Fixed Cells above.

1. Fix and permeabilize cells according to your standard protocol.
2. Dilute NucSpot® Stain, 1000X in DMSO to a final concentration of 1X in PBS or similar buffer. For example, add 1 uL of NucSpot® 1000X stock to 1 mL of buffer and vortex to mix well.
3. Incubate the sample with diluted NucSpot® Stain for 10 minutes or longer at room temperature, protected from light. Staining intensity will continue to increase with longer incubation times. Washing before imaging is optional but not required.
4. Image fluorescence in the appropriate detection channel (see Table 1).

Selective staining of dead cells in live cultures

Note: See Considerations for Live/Dead Discrimination. Protocols are provided below for selective dead cell staining by media exchange, or by direct addition of a 10X staining solution to the medium already on the cells. The direct addition method is convenient because it does not require removal of cell culture medium, which can result in loss of floating dead cells. It also eliminates the need to centrifuge suspension cells before staining.

Cell staining by media exchange:

1. Dilute NucSpot® Stain, 1000X in DMSO, to a final concentration of 1X in complete cell culture medium or buffer of your choice. For example, add 1 uL of NucSpot® 1000X stock to 1 mL of medium or buffer and vortex to mix well.
2. Remove the culture medium from the cells and replace it with the medium containing NucSpot® Stain prepared in step 1.
3. Incubate cells for 15 minutes or longer at room temperature or 37°C, protected from light.
4. Analyze cells by microscopy or flow cytometry in the appropriate detection channel (see Table 1).

Cell staining by direct addition of 10X stain:

Note: To avoid a transient high concentration of DMSO or stain, we do not recommend adding undiluted (1000X) NucSpot® stock solution directly to cells.

1. Prepare 10X NucSpot® staining solution in culture medium or buffer. For example, to stain cells with a final concentration of 1X NucSpot® Stain, add 1 uL of NucSpot® 1000X stock to 100 uL of medium or buffer and vortex to mix well.
2. Without removing the medium from the cells, add 10X staining solution to the cells at a volume equal to 1/10 the total volume of the medium. For example, add 10 uL of 10X staining solution to cells in 100 uL of medium. Scale volumes proportionally as needed.
3. Immediately mix by gently pipetting half the volume of the well up and down several times, taking care not to introduce bubbles.
4. Incubate the cells for 15 minutes or longer at room temperature or 37°C, protected from light.
5. Image in the appropriate detection channel (see Table 1).

Flow cytometry analysis of DNA content (cell cycle profiling) with NucSpot® 470 or NucSpot® Far-Red

Note: Not all NucSpot® Stains are suitable for DNA content analysis, see Table 1 for validated applications.

Materials required but not provided

- Flow Cytometry Fixation/Permeabilization Kit (Cat. No. 23006, see Related Products)

1. Adjust cell density to 10^7 cells per mL and aliquot 100 uL per flow tube.
2. Fix and permeabilize cells according to the protocol for the Flow Cytometry Fixation/Permeabilization Kit (Cat. No. 23006), or use your preferred method.
3. Pellet the cells by centrifugation and wash with 1X PBS or your preferred FACS buffer.
4. Resuspend the cells in 125 uL buffer. Add 1 uL NucSpot® Stain (8X concentration, or 1:125 dilution) per tube and mix by gentle vortexing.

   Notes:

   a. A high stain concentration ensures stoichiometric DNA binding.

   b. If 0.1% Triton® X-100 or Biotium's Permeabilization Buffer (Cat. No. 22016 or kit 23006) is used, NucSpot® Stain can be included in the permeabilization buffer. For other fixation/permeabilization methods, perform a separate staining step in buffer after permeabilization.
5. Incubate 15 minutes at room temperature, protected from light.
6. Add 400 uL PBS or FACS buffer per tube. Analyze by flow cytometry in the appropriate detection channel (see Table 1). Use a linear scale for fluorescence detection and acquire data with a slow flow rate (~12 uL/minute).

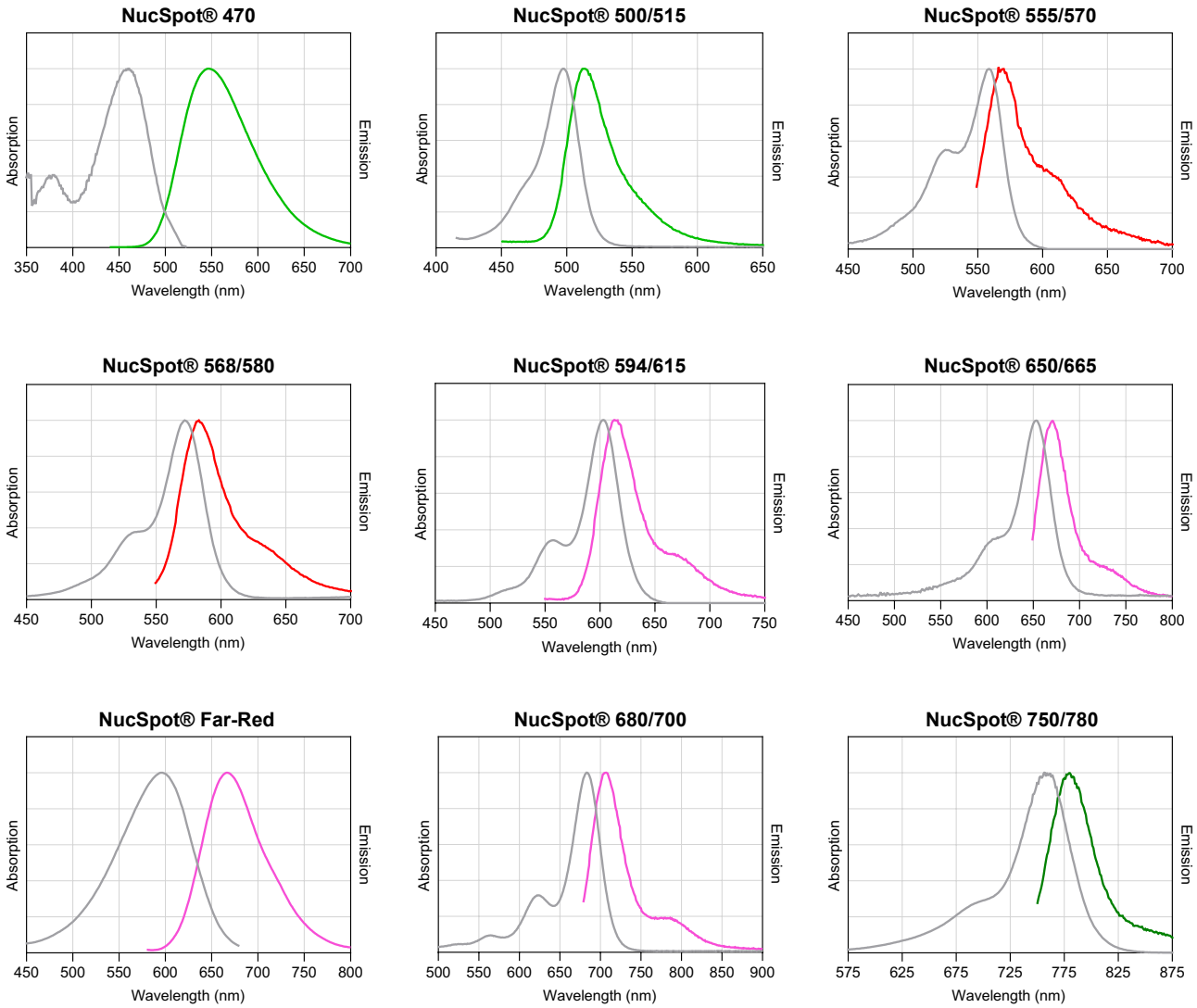

Figure 1. NucSpot® Nuclear Stains absorbance and emission spectra (with DNA).

Related Products

Cat. No.	Product
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
23016	EverBrite™ Hardset with NucSpot® 640
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23017	EverBrite TrueBlack® Hardset Mounting Medium
23018	EverBrite TrueBlack® Hardset with DAPI
23019	EverBrite TrueBlack® Hardset with NucSpot® 640
23005	CoverGrip™ Coverslip Sealant
70058... 70086	LysoView™ Lysosomal Dyes
70054... 70075	MitoView™ Mitochondrial Dyes
30050... 30086	ViaFluor® SE Cell Proliferation Kits
70062	ViaFluor® 488 Live Cell Microtubule Stain
70063	ViaFluor® 647 Live Cell Microtubule Stain
32010	Live-or-Dye NucFix™ Red Staining Kit
40009... 40043	DAPI
40044-40047	Hoechst
40016... 40048	Propidium Iodide (PI)
40037, 40084	7-AAD
40010, 40014	Ethidium Homodimer I (EthD-I)
40050, 40051	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 a wide range of cell and organelle stains, fluorescent CF® Dye antibody conjugates, apoptosis reagents, and kits for cell biology research.