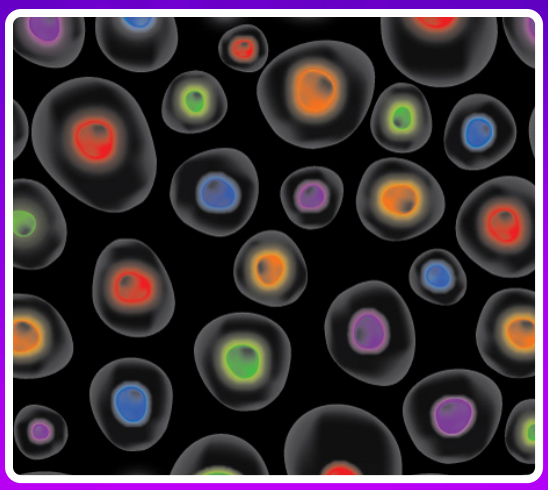


Nuclear Staining

Wide selection of novel and classic stains for microscopy or flow cytometry

Bright & Specific Nuclear Counterstains from Green to Near-IR

NucSpot® Nuclear Stains are bright and specific counterstains for fixed cells in a variety of colors from green to near-infrared (near-IR) (Figure 1). The stains have minimal fluorescence until they bind to DNA and can be used for no-wash nuclear staining. Unlike other nucleic acid 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 can be used for selective staining of dead cells in unfixed cell cultures for analysis by flow cytometry or fluorescence imaging. Several of the stains can be continuously incubated with cells for multi-day imaging (see table on page 2). NucSpot® 470 and NucSpot® Far-Red can be used for DNA content analysis of cell cycle by flow cytometry in fixed and permeabilized cells.

Features of NucSpot® Nuclear Stains

- Bright, specific staining, no RNase treatment needed
- Available in 8 colors from green to near-IR
- Selectively stain dead cells in live culture
- Minimal fluorescence until they bind to DNA
- 10 minute incubation, no wash required
- For microscopy, flow cytometry, or cell cycle profiling

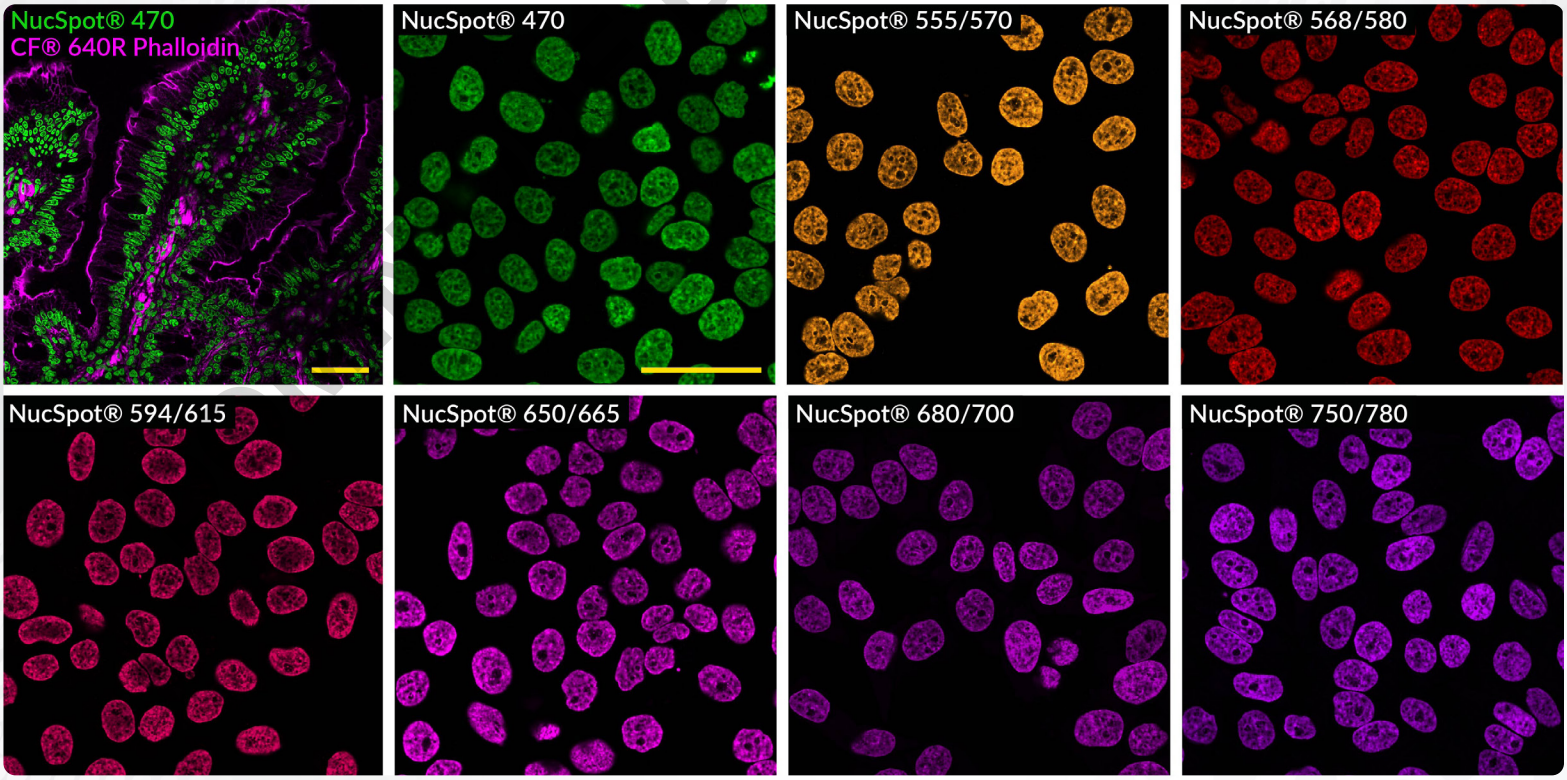


Figure 1. Top left: Intestine section stained with CF®640R Phalloidin (magenta, Cat. No. 00050) and NucSpot® 470 (green). All other images: PFA-fixed, Triton® X-100 permeabilized HeLa cells stained with NucSpot® Nuclear Stains in PBS and imaged by confocal microscopy without a wash step. All stains were used at 1X concentration except for NucSpot® 750/800, which was used at 5X concentration in order to image using 640 nm excitation. Scale bars = 50 um.

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NucSpot® Nuclear Stain Options for Fixed Cells or Dead Cells in Culture

Cat. No.	Size	NucSpot® Stain	Color (Ex/Em*)	Detection Channel	Validated Applications			
					Nuclear Counterstaining	Live/Dead Discrimination	Multi-Day Imaging	Cell Cycle Profiling‡
40083-T	20 uL	NucSpot® 470	Green (461/547 nm)	FITC	Yes	Yes	No	Yes
40083	200 uL							
41033-T	20 uL	NucSpot® 555/570	Red (559/566 nm)	Cy®3 or PE§	Yes	Yes	No	No
41033	100 uL							
41036-T	20 uL	NucSpot® 568/580	Red (572/583 nm)	Cy®3 or PE§	Yes	Yes	Yes	No
41036	100 uL							
41037-T	20 uL	NucSpot® 594/615	Red (603/613 nm)	Texas Red® or PE-Texas Red®§	Yes	Yes	Yes	No
41037	100 uL							
41034-T	20 uL	NucSpot® 650/665	Far-red (653/671 nm)	Cy®5 or APC§	Yes	Yes	No	No
41034	100 uL							
40085-T	50 uL	NucSpot® Far-Red	Far-red (597/667 nm)	PE-Cy®5 or APC †	No†	Yes (flow cytometry)†	No	Yes
40085	500 uL							
41035-T	20 uL	NucSpot® 680/700	Near-IR (683/707 nm)	Cy®5.5§	Yes	Yes	No	No
41035	100 uL							
41038-T	20 uL	NucSpot® 750/780	Near-IR (757/780 nm)	Cy®7 or APC-Cy®7§	Yes	Yes	Yes	No
41038	100 uL							

* With DNA.
§ 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.
‡ By flow cytometry.

FOR DUTSCHER SAS

Bright Nuclear-Specific Stains for Live-Cell Imaging

NucSpot® Live Nuclear Stains are cell membrane-permeant stains that specifically stain the nuclei in live or fixed cells with no need for washing. The stains are designed to have low cytotoxicity, allowing multi-day imaging up to 72 hours (Figure 2). NucSpot® Live 650 dye is also compatible with super-resolution imaging by SIM, STED, and STORM. The stains are supplied as 1000X stock solutions in DMSO. A vial of the efflux inhibitor verapamil is included as an optional additive to increase probe retention and staining, which may be helpful in some cell types.

Features of NucSpot® Live Nuclear Stains

- No-wash, nuclear-specific DNA stains
- Low toxicity for real-time live cell imaging
- Fix before or after labeling
- NucSpot® Live 650 is SIM, STED, or STORM compatible

Live Cell Imaging with NucSpot® Live 488

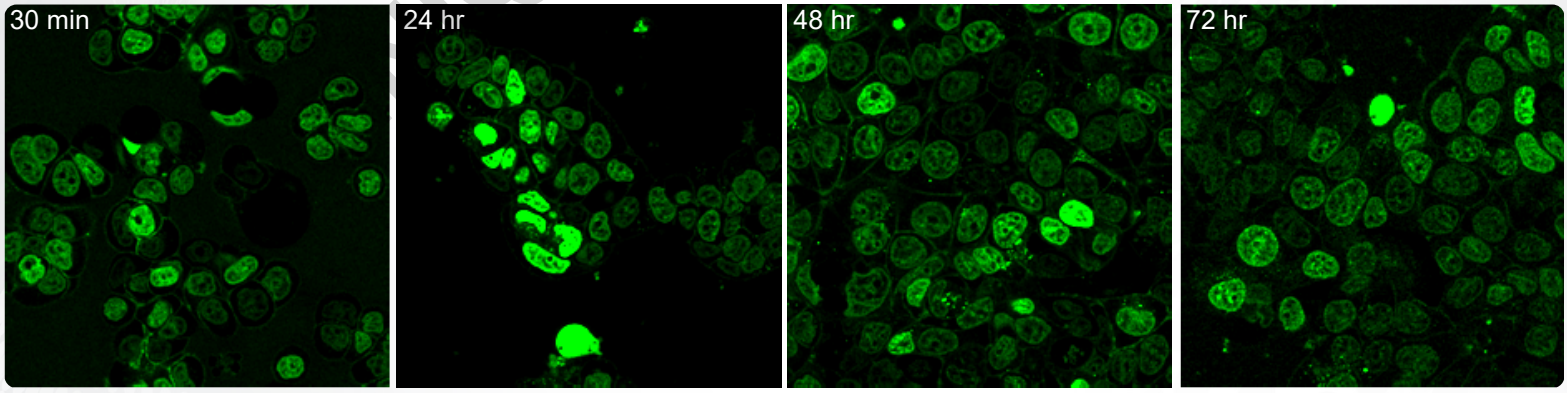


Figure 2. Timecourse of live MCF-7 cell staining with NucSpot® Live 488. Dye was added at time 0 and incubated with cells continuously for three days. NucSpot® Live 488 showed no obvious toxicity in MCF-7 cells over the course of 72 hours. With longer incubation times, some extranuclear staining can be observed.

NucSpot® Live Nuclear Stains

Product Name	Color (Ex/Em*)	Size	Cat. No.
NucSpot® 488 Live Nuclear Stain, 1000X in DMSO	Green (503/518 nm)	10 uL	40081-T
		50 uL	40081
NucSpot® 650 Live Nuclear Stain, 1000X in DMSO	Far-red (655/681 nm)	10 uL	40082-T
		50 uL	40082

* With DNA

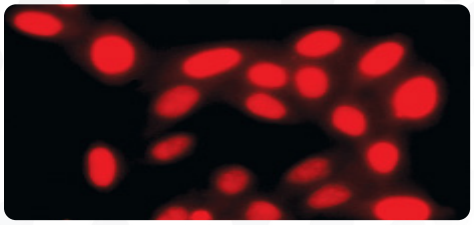


Figure 3. Live HeLa cells stained with RedDot™1 for 5 minutes at 37°C.

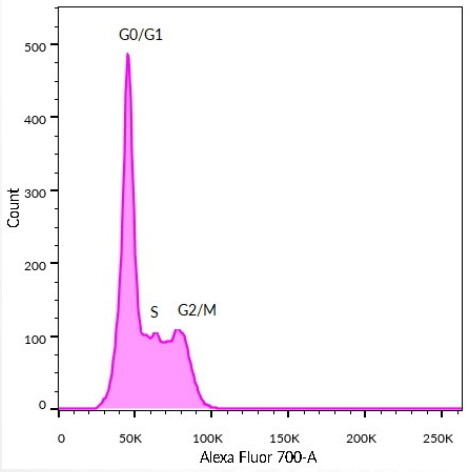


Figure 4. RedDot™1 staining for cell cycle distribution analysis. Live Jurkat cells were stained with 1X RedDot™1 for 30 minutes, then analyzed using a BD LSRII flow cytometer with 633 nm excitation and 710/50 BP filter.

RedDot™ Nuclear Stains

RedDot™1 and RedDot™2 are far-red nuclear counterstains. Their fluorescence emission is well separated from the emission peaks of red, green, and blue fluorescent probes, making RedDot™ stains ideal for multicolor imaging.

RedDot™1

Cell membrane-permeant RedDot™1 stains the nuclei of live cells rapidly and specifically (Figure 3) with a spectral profile similar to Draq5™. Validated applications for RedDot™ 1 include staining nuclei in live organisms and cell cycle analysis by flow cytometry (Figure 4). Note: For long-term live cell imaging experiments, we recommend our NucSpot® Live Stains.

RedDot™2

Cell membrane-impermeant RedDot™2 has excellent selectivity for dead cells. Our NucView®488 and RedDot™2 Apoptosis & Necrosis Kit pairs RedDot™2 with NucView®488 Caspase-3 Substrate for detection of apoptotic and necrotic cells. Unlike Draq7™, RedDot™2 staining is nuclear specific and provides excellent counterstaining without special blocking steps (Figure 5). RedDot™2 has been validated for tissue clearing protocols such as CUBIC.

RedDot™2 vs. Draq7™

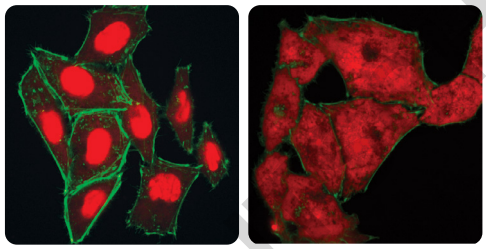


Figure 5. Fixed and permeabilized HeLa cells stained with RedDot™2 (left) or Draq7™ (right). RedDot™2 staining is highly selective for the nucleus, while Draq7™ sometimes stains both the nucleus and cytoplasm unless a separate blocking step is performed.

RedDot™1

- Cell-permeant far-red dye, similar to Draq5™
- Suitable for short-term imaging of nuclei in live cells
- Can be used for cell cycle analysis by flow cytometry

RedDot™2

- Cell-impermeant far-red dye, similar to Draq7™
- For fixed cells or tissues, or selective dead-cell staining
- Superior nuclear specificity without RNase

RedDot™1 and RedDot™2 Far-Red Nuclear Stains

Product Name	Color (Ex/Em)*	Live/Fixed	Size	Cat. No.
RedDot™1 Far-Red Nuclear Stain, 200X in H_2O	608,649 §/679 nm	Live cells only	25 uL	40060-T
			250 uL	40060
			1 mL	40060-1
RedDot™2 Far-Red Nuclear Stain, 200X in DMSO	618,665 §/698 nm	Fixed/permeabilized cells or tissues, or dead cell staining	25 uL	40061-T
			250 uL	40061
			1 mL	40061-1
NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit	618,665 §/698 nm	Apoptotic/necrotic staining in live cultures	100 assays	30072

* With DNA
§ RedDot™1 and RedDot™2 have dual excitation peaks.

A Fixable Nuclear-Specific Dead Cell Stain

Our Live-or-Dye NucFix™ Red is a unique, cell membrane-impermeant stain that covalently stains the nuclei of dead cells and can be fixed. The cells can be detected in flow cytometry or microscopy (Figure 6).

Live-or-Dye NucFix™ Red Dead Cell Stain

Product Name	Size	Cat. No.
Live-or-Dye NucFix™ Red Staining Kit	50 assays	32010-T
	200 assays	32010

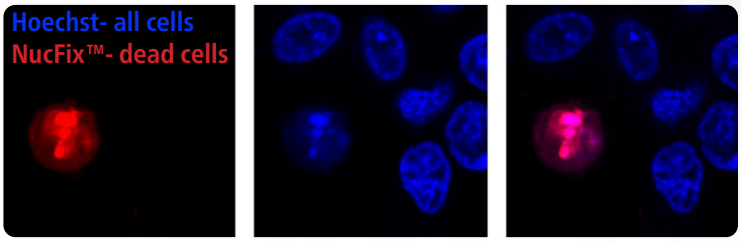


Figure 6. HeLa cells were treated with 5% ethanol for 10 minutes to kill a subset of the cells and then stained according to the product protocol with Live-or-Dye NucFix™ dead cell stain (red). Cells were also stained with Hoechst (blue) to label the nuclei of both live and dead cells and imaged on a Zeiss LSM 700 confocal microscope (NucFix™ Red in the Cy®3 channel, Hoechst in the DAPI channel). Only dead cells show bright, red nuclear staining with NucFix™.

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Other Dead Cell-Selective Nuclear Stains

For additional options for no-wash staining of dead cells in live cultures, consider our selection of cyanine-based nuclear stains.

Cyanine-based Nuclear Stain Options

Product Name	Equivalent	Color (Ex/Em)	Cat. No.
Oxazole Blue, 1 mM in DMSO	PO-PRO™-1	Blue (434/457 nm)	40091
Oxazole Blue Homodimer, 1 mM in DMSO	POPO™-1	Blue (433/457 nm)	40093
Oxazole Yellow, 1 mM in DMSO	YO-PRO®-1	Green (491/506 nm)	40089
Oxazole Yellow Homodimer, 1 mM in DMSO	YOYO®-1	Green (491/508 nm)	40090
TO Iodide, 1 mM in DMSO	TO-PRO®-1	Green (515/531 nm)	40088
Thiazole Orange Homodimer, 1 mM in DMSO	TOTO®-1	Green (514/531 nm)	40079
Oxazole Red, 1 mM in DMSO	YO-PRO®-3	Far-red (613/629 nm)	40105
Oxazole Red Homodimer, 1 mM in DMSO	YOYO®-3	Far-red (612/631 nm)	40106
Thiazole Red, 1 mM in DMSO	TO-PRO®-3	Far-red (642/657 nm)	40087
Thiazole Red Homodimer, 1 mM in DMSO	TOTO®-3	Far-red (642/661 nm)	40080

Cyanine-based Nuclear Stains

- Cell membrane-impermeant and dead cell selective nuclear stains
- Bright and sensitive staining
- No-wash staining

Ethidium Homodimers-I and -III

Ethidium Homodimer I binds to both DNA and RNA in a sequence-independent manner and with a >30-fold fluorescence enhancement. Ethidium Homodimer III was developed by Biotium as a brighter alternative to EthD-I.

Ethidium Homodimers-I and -III

Product Name	Color (Ex/Em)*	Size	Cat. No.
Ethidium Homodimer I (EthD-I)	Red (527/624 nm)	1 mg	40010
		0.5 mL (2 mM in DMSO)	40014
Ethidium Homodimer III (EthD-III)	Red (532/625 nm)	1 mg	40050
		200 uL (1 mM in DMSO)	40051

* With DNA, Ethidium Homodimers I and III also have a strong UV absorbance peak at 279 nm

Ethidium Homodimers-I and -III

- Membrane-impermeant red nuclear stains
- Brighter alternatives to PI
- EthD-III is superior to EthD-I

Classic Nuclear Stains

Biotium offers many other classic nuclear stains with high purity and competitive prices. These include the mainstay dyes Hoechst, DAPI, and PI in several formats. Visit biotium.com for more options.

Classic Nuclear Stain Options

Product Name	Size	Cat. No.
7-AAD (7-aminoactinomycin D)	1 mg	40037
7-AAD (7-aminoactinomycin D), 1 mg/mL	1 mL	40084
Hoechst 33342, 10 mg/mL in H_2O	10 mL	40046
Hoechst 33258, 10 mg/mL in H_2O	10 mL	40044
Hoechst 33342, trihydrochloride trihydrate	100 mg	40047
Hoechst 33258, pentahydrate	100 mg	40045
DAPI in H_2O, 10 mg/mL	1 mL	40043
DAPI	10 mg	40011
DAPI, dilactate	10 mg	40009
Propidium iodide (PI)	100 mg	40016
Propidium iodide, 1 mg/mL in H_2O	10 mL	40017
Propidium iodide buffer, 50 ug/mL in PBS	2 mL	40048

EverBrite™ Mounting Media

A revolutionary antifade mounting medium formulated for preserving fluorescence of our CF® Dyes and other fluorochromes, including Alexa Fluor® and cyanine dyes. Available with DAPI and/or TrueBlack® Autofluorescence Quencher.

EverBrite™ Mounting Media

Product Name	Size	Cat. No.
EverBrite™ Mounting Medium with DAPI	10 mL	23002
EverBrite™ Hardset Mounting Medium with DAPI	10 mL	23004
Drop-n-Stain EverBrite™ Mounting Medium with DAPI	2 mL	23009-T
	10 mL	23009
EverBrite TrueBlack® Hardset with DAPI	2 mL	23018-T
	10 mL	23018

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