Far-Red Nuclear-Specific Dyes for Cell Counterstaining

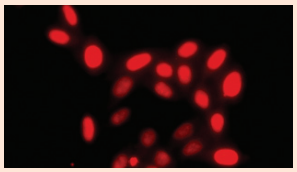


Figure 2. Live HeLa cells stained with 1X RedDot™1 for 5 minutes at 37°C.

RedDot™1 and RedDot™2 are two far-red DNA-binding dyes designed as nuclear counterstains for live or fixed and permeabilized cells, respectively.

RedDot™ dyes combine the advantages of existing nuclear counterstains, such as DAPI, Draq™5 and Draq™7, with some key advantages. Spectrally similar to Draq™5 and Draq™7, the RedDot™ dyes are excitable by several common laser lines and emit fluorescence in the far-red spectral region. RedDot™ fluorescence emission is well separated from the emission peaks of other popular fluorescent probes (Figure 1), making RedDot™ dyes ideal counterstains for multicolor imaging.

Cell permeable RedDot™1 stains the nuclei of live cells rapidly and specifically (Figure 2).

Cell membrane-impermeable 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 Draq™5 and Draq™7, which show significant cytoplasmic staining in permeabilzed cells unless additional blocking steps are performed, RedDot™2 staining is highly selective for the nucleus. Thus, RedDot™2 provides excellent nuclear counterstaining in fixed and permeabilized cells (Figure 3).

RedDot™ dyes can be used to stain adherent or suspension cells and tissue sections. The dyes are highly thermostable and photostable, providing convenient handling and ideal for demanding applications such as confocal microscopy. Selected applications are listed on page 2.

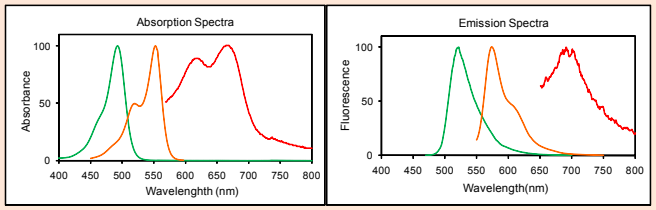


Figure 1. Absorption and emission spectra of FITC, Cy™3 and RedDot™2 in the presence of DNA. RedDot™1 and RedDot™2 have similar spectra.

Two Far-Red Nuclear Dyes for All of Your Counterstaining Needs

RedDot™1: A far-red cell membrane-permeable nuclear dye for staining the nuclei of live cells.

RedDot™2: A far-red cell membrane-impermeable nuclear stain for selective dead cells staining, or nuclear counterstaining of fixed and permeabilized cells and tissue sections.

US Orders:
1-800-304-5357

Far-red nuclear counterstains

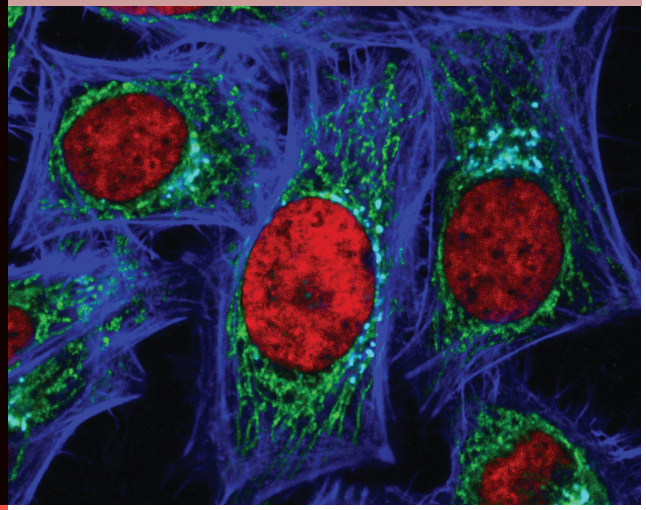
RedDot™1

For live cell nuclear staining

&

RedDot™2

For nucleus-specific counterstaining of fixed cells and tissues



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Fluorescent Tools for Cell Biology

Two RedDot™ Dyes, Many Possible Applications

Microscopy

RedDot™ dyes are superior nuclear counterstains due to their high specificity, excellent photostability and far-red emission (~680 nm) that is well separated from the emission maxima of other common fluorophores. The dyes can be excited by several laser lines, including 488, 532, 543, 568, 594, 633, 635 and 647 nm laser lines. These properties make RedDot™ dyes useful tools for immunocytochemistry studies and high-content screening assays.

RedDot™2 vs. Draq™7

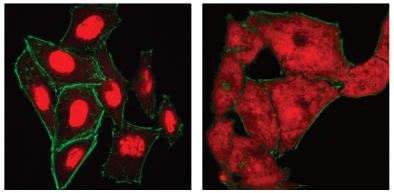

Figure 3. Formaldehyde fixed and detergent permeabilized HeLa cells stained with 1X RedDot2 (left) or 3 uM Draq7 (right) in PBS for 10 minutes. RedDot2 staining is highly selective for the nucleus, while Draq7 stains the nucleus and cytoplasm unless a separate blocking step is performed. Actin filaments are stained with CF488A phalloidin (green).

Flow Cytometry

RedDot™1 can be used for analysis of cell cycle distribution. The dye can be excited by a number of laser lines, including 488, 532, 543, 568, 594, 633, 635, 640, and 647 nm lines, with emission in the far-red channel.

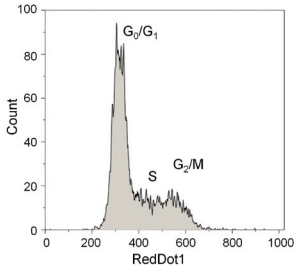

Figure 4. RedDot1 staining for cell cycle distribution analysis. Live Jurkat cells were stained with 1X RedDot1 for 30 minutes at 37°C, then analyzed using a BD LSRII flow cytometer with 633 nm excitation and 710/50 BP emission filter. Image courtesy of Philip Hexley, Shriners Flow Cytometry Core Facility, Shriners Hospital for Children and University of Cincinnati.

Other Applications

RedDot™ dyes can be used for normalizing or counting cell number using fluorescence plate readers or the LI-COR Odyssey® near-IR system for In-Cell Western™.

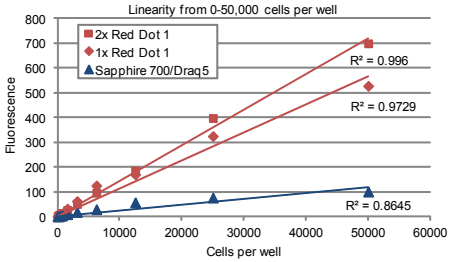

Figure 5. RedDot1 staining of HeLa cells for cell number normalization. HeLa cells were seeded in 96 wells at the indicated densities. After 24 hours, cells were fixed, permeabilized, and stained with the indicated dyes for one hour at room temperature according to the supplier's protocol for DRAQ5/Sapphire700. Fluorescence was quantitated using the LI-COR Odyssey system. HeLa cells seeded at 25,000 cells per well were confluent at the time of assay.

RedDot™

Ordering information

Product Name	Product features	Cat. #	Unit Size
RedDot™1, 200X in water	For live cell nuclear staining	40060-T	25 uL trial size
		40060	250 uL
		40060-1	1 mL
RedDot™2, 200X in DMSO	For selective staining of dead cells, or nuclear counterstaining of fixed and permeabilized cells	40061-T	25 uL trial size
		40061	250 uL
		40061-1	1 mL
NucView™488 and RedDot™2 Apoptosis & Necrosis Kit	Stain apoptotic cells green and necrotic/late apoptotic cells far red for fluorescence microscopy or flow cytometry	30072	100 assays

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