Revised: October 23, 2018

Product Information

RedDot™1

Catalog #	Unit Size	Concentration	Storage
40060-T	25 µl	200X in water	Store at -20°C, protect from light
40060	250 µl		
40060-1	1 ml		

Storage and Handling

Store RedDot™1 at 4°C or -20°C, protected from light. RedDot™1 is stable and can tolerate multiple rounds of freeze thaw. When stored as directed, RedDot™1 is stable for at least one year from the date of receipt.

CAUTION: RedDot™1 may be toxic and mutagenic. Handle with care. Dispose of RedDot™1 as toxic waste according to your institution's regulations.

Spectral properties

Abs/Em maxima with DNA: 662/694 nm
RedDot™1 can be excited at a wide range of wavelengths including the 488 nm laser for flow cytometry and by wavelengths up to 647 nm.

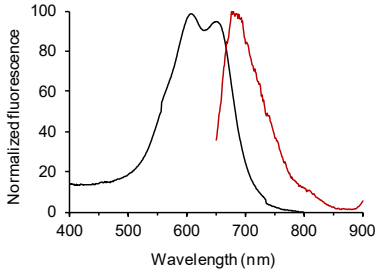

Figure 1. Absorption and emission spectra of RedDot™1 in live Jurkat cells.

Product Description

RedDot™1 is a far red nuclear stain for live cells. RedDot™1 dye can be excited by wavelengths from 488 to 647 nm and emits far red fluorescence with emission maximum at 694 nm (Fig. 1). RedDot™1 specifically stains the nucleus of live cells (Fig. 2). RedDot™1 staining does not require a wash step and demonstrates greater photostability than the traditional blue fluorescent nuclear stains DAPI and Hoechst. While DAPI and Hoechst dyes show a strong preference for A-T rich regions, RedDot™1 is relatively insensitive to sequence base composition.

RedDot™1 nuclear staining is not fixable. RedDot™1 is not recommended as a nuclear counterstain for microscopic imaging of fixed and permeabilized cells. RedDot™2 (see related products) is recommended for nuclear-specific counterstaining of fixed and permeabilized cells and tissue sections.

RedDot™1 can be used to stain fixed and permeabilized cells for cell number normalization for In-Cell Western™ assays using a near-infrared scanner such as the LI-COR® Odyssey®. In fixed cells, RedDot™1 staining generates a linear fluorescence signal in the 700 nm channel in proportion to cell number. In a direct comparison, RedDot™1 staining for cell normalization showed higher signal and a wider linear range compared to DRAQ5™/Sapphire700™ staining (Fig. 3).

RedDot™1 also can be used to stain live cells for cell cycle distribution analysis by flow cytometry (Fig. 4).

Staining Protocols

Live cell nuclear staining

Because RedDot™1 staining does not require a wash step, it can be added after other cell labeling or treatment and immediately prior to fluorescence analysis.

1. Dilute RedDot™1 dye in cell culture medium or PBS to a final concentration of 1X.
2. Incubate cells with medium or PBS containing RedDot™1 for 5-30 minutes at room temperature or for 5 minutes at 37°C.
3. Detect far red nuclear staining by fluorescence microscopy, flow cytometry, or fluorescence microplate reader.

   Note: Like many membrane permeable nucleic acid binding dyes, RedDot™1 demonstrates cellular toxicity within 4-18 hours after staining (toxicity may vary by cell type).

   Note: For flow cytometry analysis of cell cycle distribution, use a slow flow rate and linear scaling for fluorescence acquisition.

Staining of fixed and permeabilized cells for cell number normalization

We recommend performing a standard curve of cell density and testing RedDot™1 at 1X and 2X final concentration to determine optimal staining concentration for linear fluorescence signal for your cell type (see Fig. 3).

1. Fix cells in 4% formaldehyde in phosphate buffered saline (PBS) for 15 minutes at room temperature or 30 minutes at 4°C.
   Note: Alternatively, cells can be fixed with methanol for 5-10 minutes at -20°C.
2. Permeabilize and block cells by incubating with the immunofluorescence blocking buffer of your choice containing 0.1% Triton X-100 for 30 minutes at room temperature.
3. Optional: perform immunofluorescence staining according to your standard protocol.
4. Dilute RedDot™1 in PBS to a final concentration of 1X or 2X. Stain cells for 10 minutes or longer at room temperature.
   Note: RedDot™1 can be diluted in immunofluorescence blocking buffer and incubated together with antibodies during immunofluorescence staining.
5. Optional: wash cells in PBS according to your immunostaining protocol.
6. For LI-COR® Odyssey® near-IR fluorescent readers, remove buffer and allow wells to dry before imaging. Store the dry plate at 4°C protected from light.
7. Read fluorescence in the 700 nm channel according to the instrument manufacturer's instructions.

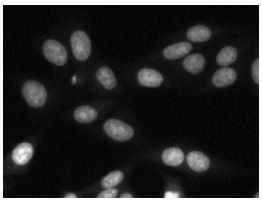

Figure 2. RedDot™1 staining of live HeLa cells.

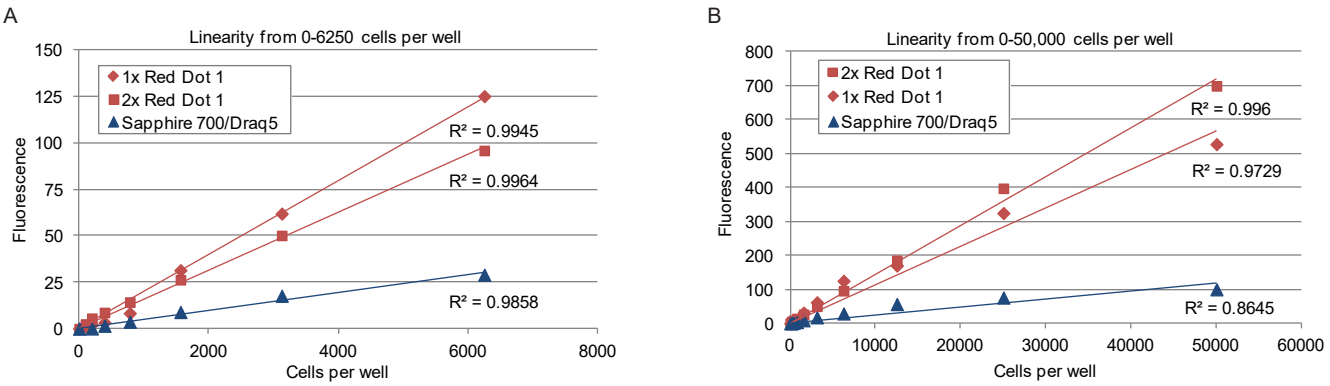

Figure 3. RedDot™1 staining of HeLa cells for cell number normalization. HeLa cells were seeded in 96 wells at the indicated densities. After 24 hours, cells were fixed, permeabilized, and stained with the indicated dyes for one hour at room temperature according to the supplier's protocol for DRAQ5™/Sapphire700™. A. Linearity of fluorescence signal for 0-6250 cells per well. B. Linearity of fluorescence signal for 0-50,000 cells per well. HeLa cells seeded at 25,000 cells per well were confluent at the time of assay.

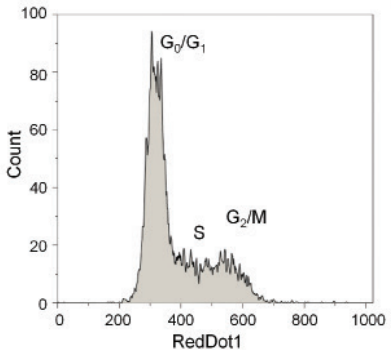

Figure 4. RedDot™1 staining for cell cycle distribution analysis. Live Jurkat cells were stained with 1X RedDot™1 for 30 minutes at 37°C, then analyzed using a BD LSRII flow cytometer with 633 nm excitation and 710/50 BP emission filter. Image courtesy of Philip Hexley, Shriners Flow Cytometry Core Facility,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

Related Products

Cat.#	Product Name	Unit Size
40061-T	RedDot™2	25 µl
40061	RedDot™2	250 µl
40061-1	RedDot™2	1 ml
30068	ViaFluor® 405-SE Cell Proliferation Kit	Kit
30050	CFDA-SE Cell Proliferation Kit	Kit
23001	EverBrite™ Mounting Medium	10 ml
23002	EverBrite™ Mounting Medium with DAPI	10 ml
40009	DAPI, dilactate	10 mg
40011	DAPI (dihydrochloride salt)	10 mg
40043	DAPI in water, 10 mg/ml	1 ml
40016	Propidium iodide	100 mg
40017	Propidium iodide in water, 1.0 mg/ml	10 ml
40048	Propidium iodide buffer, 50 µg/ml	2 ml
40044	Hoechst 33258 in water, 10 mg/ml	10 ml
40045	Hoechst 33258, pentahydrate	100 mg
40046	Hoechst 33342 in water, 10 mg/ml	10 ml
40047	Hoechst 33342, trihydrochloride trihydrate	100 mg

Please visit **www.biotium.com** to view our full selection of products featuring bright and photostable fluorescent CF® dyes, including secondary antibodies and Mix-n-Stain™ antibody labeling kits. Biotium also offers a variety of apoptosis and cell viability assays for flow cytometry analysis, including mitochondrial membrane potential dyes, fluorescent Annexin V conjugates, and NucView®488 Caspase-3 Substrate for live cells.