Far-Red Nuclear-Specific Dyes for Cell Counterstaining

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 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 permeabilized 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.

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
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, 640, 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

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.

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™.

Ordering information

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

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).

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.

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™1, RedDot™2, and CF™ dyes are trademarks of Biotium, Inc.; DRAQ5™ and Draq7™ are trademarks of Biostatus Limited; Odyssey®, In Cell Western™, and Sapphire700™ are trademarks of LI-COR Biosciences; Cy™3 and Cy™5 are trademarks of GE Healthcare.