**Product Information**

- **Material Number:** 564903
- **Size:** 50 µL
- **Concentration:** 5 mM

**Description**

DRAQ5™ (Deep Red Anthraquinone 5) is a far-red fluorescent DNA dye. DRAQ5™ is cell permeable and may be used to stain nucleic acids in live or fixed cells for cell cycle analysis by DNA content, nuclear visualization, or discrimination of nucleated cells from debris or enucleated cells. DRAQ5™ has an excitation wavelength maxima of 598/646 nm, but can also be sub-optimally excited by the 488 nm laser. Its emission wavelength maximum is 681 nm, or 697 nm when intercalated with double stranded DNA.

**Panel 1. Cell cycle analysis of stimulated mouse splenocytes.** Mouse splenic leukocytes (SPL) were cultured (3 days) without (Left Plot) or with (Right Plot) Purified NA/LE Hamster Anti-Mouse CD3ε (Cat. No. 553057) and Purified NA/LE Hamster Anti-Mouse CD28 (Cat. No. 553294) antibodies. Cells were harvested, stained with Fixable Viability Stain 450 (Cat. No. 562247) and stained for BrdU and DNA content with DRAQ5™ according to the FITC BrdU Flow Kit Manual (Cat. No. 557891/559619). Viable cells based on Fixable Viability Stain 450 staining were analyzed for BrdU staining and DNA content. Stimulated cells show a large increase in S-phase (BrdU+, DRAQ5™ 2N-4N) and G2/M-phase (BrdU-, DRAQ5™ 4N) cells. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.

**Panel 2. Flow cytometric analysis of HeLa cell DNA content.** HeLa cells in log phase growth were harvested using Gibco® Cell Dissociation Buffer (Life Technologies) and resuspended in complete medium containing 20 µM DRAQ5™ (15 min, 37°C). Cells were pelleted by centrifugation, supernatant was aspirated, and cells were resuspended in DPBS for DNA content analysis using a BD LSRFortessa™ Cell Analyzer System. Histograms were deconvoluted by FlowJo™ software into G0/G1, S, and G2/M peaks.

**Panel 3. Immunofluorescence analysis of E-cadherin expression in human MCF7 cells.** Cultured cells from the MCF-7 (Breast adenocarcinoma, ATCC HTB-22) cell line were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), permeabilized with ice-cold BD Phosflow™ Perm Buffer III (Cat. No. 558050), washed and stained with Purified Mouse Anti-E-Cadherin antibody (Cat. No. 610181/610182) at 5 µg/mL. After washing, cells were stained with the second step reagent, BD Horizon™ BV421 Goat Anti-Mouse Ig (Cat. No. 568346) at 1.5 µg/mL (pseudo-colored green) and DRAQ5™ (pseudo-colored red) as a nuclear counterstain. The image was captured on a Zeiss LSM 710 Confocal Microscope and merged using ImageJ software.

**Preparation and Storage**

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

**Application Notes**

**Application**

<table>
<thead>
<tr>
<th>Application</th>
<th>Tested During Development</th>
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</thead>
<tbody>
<tr>
<td>Bioimaging</td>
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<tr>
<td>Immunofluorescence</td>
<td></td>
</tr>
<tr>
<td>Intracellular staining (flow cytometry)</td>
<td>Tested During Development</td>
</tr>
</tbody>
</table>

**Recommended Assay Procedure:**

**Staining of Live Cells for DNA Content Analysis by Flow Cytometry**

1. Obtain a single cell suspension.
2. Resuspend cells at 0.5 x 10^6 cells/mL or less in complete medium or other azide-free buffer containing 20 µM DRAQ5™.
3. Incubate at 37°C for 5-15 minutes.
a. The optimal cell density, concentration of DRAQ5™, and stain time for DNA content analysis may vary by cell type. Assay conditions should be optimized in early experiments for best results.

4. Pellet cells by centrifugation and aspirate medium or other azide-free buffer containing DRAQ5™.
5. Resuspend cells in BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 1× PBS and immediately proceed to analysis by flow cytometry. Cells may also be analyzed without washing, but this may decrease DNA content histogram resolution.

### Staining of Fixed Cells for DNA Content Analysis by Flow Cytometry

1. Obtain a single cell suspension.
2. Treat cells on ice for 30 minutes with 70-80% ice-cold ethanol.
   a. Ethanol fixation typically provides the most resolved histograms. However, this reagent has also been successfully used for DNA content analysis with the Transcription Factor Buffer Set (Cat. No. 562574) or BD Cytofix™ Fixation Buffer (Cat. No. 554655) and BD Phosflow™ Perm III (Cat. No. 558050) protocol.
3. Wash cells once with BD Pharmingen™ Stain Buffer (FBS).
4. Dilute DRAQ5™ solution to 20 μM in 1× Dulbecco's Phosphate Buffered Saline (DPBS) or other azide-free buffer immediately prior to use.
5. Stain cells for 5-15 minutes at a cell density of 0.5 x 10^6 cells/mL or less. No further wash is necessary prior to analysis.
   a. The optimal cell density and concentration of DRAQ5™ for DNA content analysis may vary by cell type. Assay conditions should be optimized in early experiments for best results.
6. Proceed to analysis by flow cytometry.

### Immunofluorescent Staining of Live Cells for Nuclear Visualization

1. Dilute DRAQ5™ solution to 5-20 μM in complete medium or other azide-free buffer immediately prior to use.
2. Add DRAQ5™ solution to samples and incubate at 37°C for 5-30 minutes. The stain time required is cell type dependent.
3. Remove DRAQ5™ solution from cells at the end of the incubation period and add BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 1× DPBS. Cells may also be analyzed without washing, but this may increase background from unbound dye.
4. Proceed to imaging. We recommend using a 715LP or longer wavelength filter, though the dye is well-detected in filters typically used to detect Alexa Fluor® 647 (eg, 660/20 or 692/40 nm). Note that dsDNA-bound dye will fluoresce brightly in the nucleus and unbound dye may fluoresce dimly in the cytoplasm, allowing segmentation of the cytoplasmic and nuclear compartments.

### Immunofluorescent Staining of Fixed Cells for Nuclear Visualization

1. Fix and permeabilize cells as desired.
2. Dilute DRAQ5™ solution to 5-20 μM in 1× DPBS or other azide-free buffer immediately prior to use.
3. Add DRAQ5™ solution to each well at least 5 minutes before analysis.
4. Proceed to imaging. We recommend using a 715LP or longer wavelength filter, though the dye is well-detected in filters typically used to detect Alexa Fluor® 647 (eg, 660/20 or 692/40 nm). Note that dsDNA-bound dye will fluoresce brightly in the nucleus and unbound dye may fluoresce dimly in the cytoplasm, allowing segmentation of the cytoplasmic and nuclear compartments.

**Note:** This reagent has been developed and certified for the Bioimaging application. However, a routine Bioimaging test is not performed on every lot.
Warning: DRAQ5™ contains < 1% 1,5-BIS[(2-DIMETHYLAMINO)ETHYLAMINO]-4,8-DIHYDROXYANTHRACENE-9,10-DIONE

Hazard statements
Causes skin irritation.
Causes serious eye irritation.
May cause respiratory irritation.

Precautionary statements
Wear protective gloves/protective clothing/eye protection/face protection.
Wash thoroughly after handling.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.
Continue rinsing. If eye irritation persists: Get medical advice/attention.
Take off contaminated clothing and wash before reuse.
Call a POISON CENTER or doctor/physician if you feel unwell.
If skin irritation occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water
Avoid breathing dust/fume/gas/mist/vapors/spray.
Use only outdoors or in a well-ventilated area.
IF INHALED: Remove person to fresh air and keep comfortable for breathing.
Store in a well-ventilated place. Keep container tightly closed. Store locked up.
Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Suggested Companion Products

<table>
<thead>
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<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
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<td>BV421 Goat Anti-Mouse Ig</td>
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Product Notices
1. DRAQ5™ is a registered trademark of BioStatus Ltd.
2. FlowJo is a trademark of Tree Star Inc.
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.

References