Technical Data Sheet

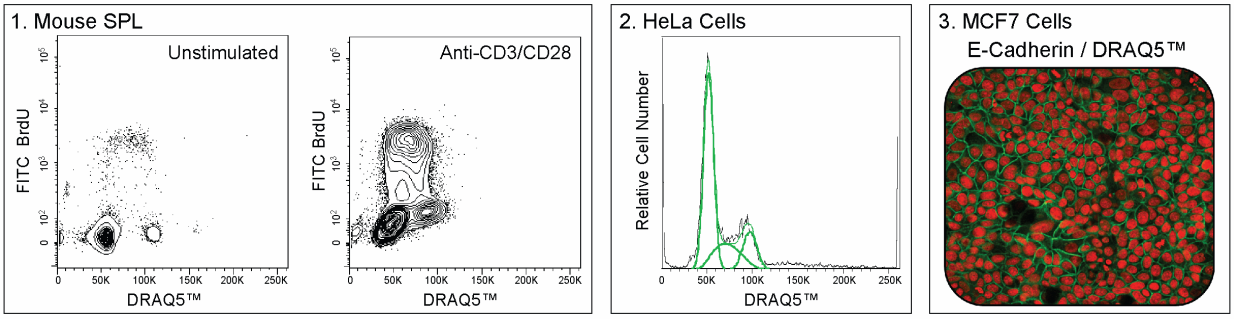
DRAQ5™

Product Information

Material Number: **564902**
Size: 200 µL

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 suboptimally 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 leucocytes (SPL) were cultured (3 days) without (Left Plot) or with (Right Plot) Purified NA/LE Hamster Anti-Mouse CD3e (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. 563846) 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

Bioimaging	Tested During Development
Immunofluorescence	Tested During Development
Intracellular staining (flow cytometry)	Tested During Development

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™.

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is stictly prohibited.
For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.
© 2017 BD. BD, the BD Logo and all other trademarks are property of Becton, Dickinson and Company.

5. Resuspend cells in BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 1× Dulbecco's Phosphate Buffered Saline (DPBS) 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× 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× PBS. 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 for the Bioimaging application. However, a routine Bioimaging test is not performed on every lot.

Warning: *DRAQ5™ contains < 1% 1,5-BIS{[2-(DIMETHYLAMINO)ETHYL]AMINO}-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

Catalog Number	Name	Size	Clone
557891	FITC BrdU Flow Kit	200 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564903	DRAQ5™	50 µL	(none)
554655	Fixation Buffer	100 mL	(none)
558050	Perm Buffer III	125 mL	(none)
562247	Fixable Viability Stain 450	0.1 mg	(none)
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
610181	Purified Mouse Anti-E-Cadherin	50 µg	36/E-Cadherin
559619	FITC BrdU Flow Kit	100 Tests	(none)
562574	Transcription Factor Buffer Set	100 Tests	(none)

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.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Akagi J, Kordon M, Zhao H, et al. Real-time cell viability assays using a new anthracycline derivative DRAQ7®. *Cytometry A.* 2013; 83(2):227-234. (Methodology)

Edward R. Red/far-red fluorescing DNA-specific anthraquinones for nucl:cyto segmentation and viability reporting in cell-based assays. *Methods Enzymol.* 2012; 505:23-45. (Methodology)

Smith PJ, Blunt N, Wiltshire M, et al. Characteristics of a novel deep red/infrared fluorescent cell-permeant DNA probe, DRAQ5, in intact human cells analyzed by flow cytometry, confocal and multiphoton microscopy. *Cytometry.* 2000; 40(4):280-291. (Methodology)

Smith PJ, Wiltshire M, Davies S, et al. A novel cell permeant and far red-fluorescing DNA probe, DRAQ5, for blood cell discrimination by flow cytometry. *J Immunol Methods.* 1999; 229(1):131-139. (Methodology)

Smith PJ, Wiltshire M, Errington RJ. DRAQ5 Labeling of Nuclear DNA in Live and Fixed Cells. *Curr Protoc Cytom.* 2004; 7(7.25)(Methodology)