ab108410 – DRAQ5™

(5 mM) Protocol

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

DRAQ5™,*1, 5– bis{[2-(di- methylamino) ethyl] amino}- 4, 8- dihydroxyanthracene-9, 10-dione*, is a cell permeable far-red fluorescent DNA dye that can be used in live or fixed cells in combination with common labels such as GFP or FITC.

DRAQ5™ can be used in flow cytometry, live cell imaging and cell-based assays and is highly compatible with existing protocols across a wide range of instrumentation platforms.

Key features of DRAQ5™ include:

- Rapid uptake into living cells, providing a high level of nuclear discrimination
- No photobleaching effect
- It can be used in most cell types, eukaryotic and prokaryotic: mammalian, bacterial, parasitic, plant, etc ...
- No compensation needed with common FITC/GFP + PE combinations in flow cytometry
- No RNase treatment required

2. Reagents Required

- ab108410: DRAQ5™ (5mM)
- Phosphate Buffered Saline (PBS), without sodium azide, or other culture medium

3. Storage and Handling

Before handling DRAQ5™ please read the MSDS supplied with the product.

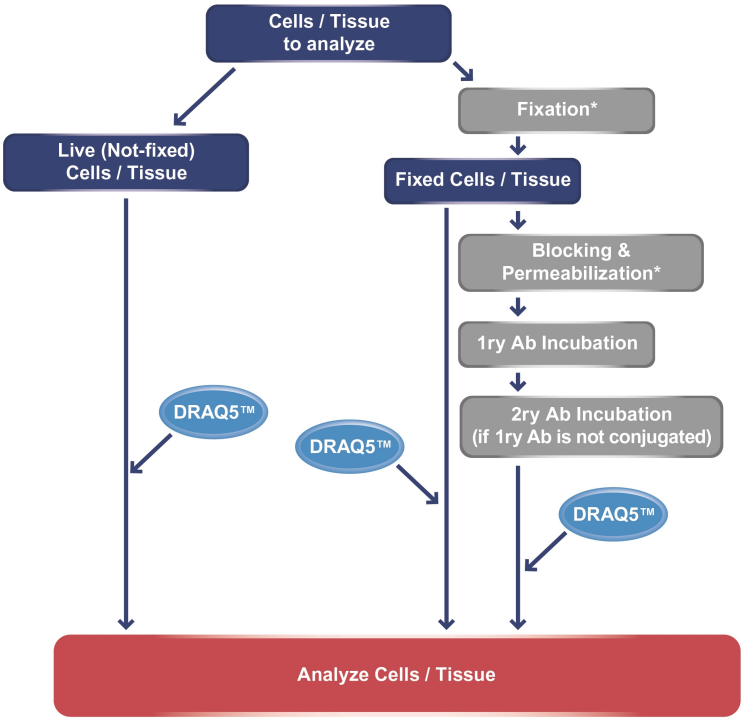
Store product at 4°C in the dark. Do not freeze.

Undiluted product is stable for at least 3 years if kept under the recommended conditions. Diluted product should be used as soon as possible.

4. Overview: Use of DRAQ5™

DRAQ5™ is added in the final staining step of a labeling procedure as there is no further washing step required.

The following chart gives an overview on where DRAQ5™ can be added depending on the type of experiment.



*optional step

5. General Protocols

Protocol I: Live cell staining with DRAQ5™ for nuclear visualization by imager or laser scanning confocal / epifluorescence microscopy.

This general protocol is a guideline, and we recommend adapting it to each user's best protocol.

DRAQ5™ is usually added at the last step of the staining procedure as no washing is required. Alternatively, it can be added to the assay media for a live cell assay.

A. Cell Preparation (and Fixation)

1. Prepare cells for staining with DRAQ5™: centrifuge and resuspend cells in appropriate buffer such as PBS (or other culture media) at a concentration of ≤ 4 x 10^5 cells/ml in a test tube.

   For adherent cells, estimate the number of cells based on confluence level or tissue section dimensions.

2. If a fixation step is required, most common fixation methods are compatible with DRAQ5™.

3. For cells expressing fluorescent proteins (e.g. GFP/YFP-tagged proteins), DRAQ5™ can be mixed with formaldehyde fixative to provide a one-step "*fix & stain*" protocol. To prepare, mix equal volumes of 10μM DRAQ5™ and 8% formaldehyde.

4. For cells requiring indirect immunostaining (primary and / or secondary antibodies) block and permeabilize accordingly to your favorite protocol. Wash thoroughly after primary antibody incubation.

B. Cell Staining

5. Add DRAQ5™ directly as supplied to a final concentration of 5μM. This will be used as an overlay for adherent cells / tissue sections, added to the chamber / well liquid directly or in fresh media following a wash step.

6. Gently mix by pipetting and then incubate for 5 – 30 minutes at room temperature.

   DRAQ5™ staining is accelerated at 37°C and incubation time may be reduced but this should be checked by titration and for each cell type.

   Note: Protect cells from light during incubation period if other fluorescent stains (such secondary antibodies) have been applied to the cells, as they may otherwise suffer photo-bleaching.

For time-lapsed assays (e.g. studying translocation of an eGFP-tagged protein), DRAQ5™ may be added to the assay medium for the duration of the assay (typically 0.5 – 3 hours) at 1µM prior to any agonist / antagonist additions.

7. Cells can be analyzed directly without further treatment or washing. Simply remove the excess liquid and apply mountant and coverslip as required.

   DRAQ5™ may be added to anti-fade mountants such as Fluoroshield Mounting Medium (Ab104135). Never use mountant containing DAPI.

C. Data Analysis

Both conventional microscopy and confocal microscopy can be used to analyze DRAQ5™-stained cells.

It is important to consider the combinations of fluorochromes and filters for the experiments.

EXCITATION:

DRAQ5™ may be sub-optically excited by wavelengths from 488 nm and up to 647 nm (Exλ_{max} 646 nm). Typically, for cell imaging, excitation is performed with either 633 nm or 647 nm wavelengths to achieve.

EMISSION:

Emission starts at 655 nm (Emλ_{max} 681 nm/ 697 nm dsDNA-bound).

Suitable filters include 695LP, 715LP or 780 LP.

DRAQ5™ has no spectral emission overlap with FITC, R-PE, GFP, YFP, DyLight® 488 or many other fluorescent tags and fluorescing proteins allowing image acquisition in one scan.

Protocol II: Cell staining with DRAQ5™ for DNA cell cycle analysis and nucleated cell "gating" by flow cytometry or by cell imaging.

This general protocol is a guideline, and we recommend adapting it to each user's best protocol.

A. Cell Preparation (and Fixation)

1. Prepare cells for staining with DRAQ5™: centrifuge and resuspend cells in appropriate buffer such as PBS (or other culture medium) at a concentration of ≤4 x 10^5 cells/ml in a test tube.

2. For cells requiring additional fluorescent staining (either conjugated-primary antibody or primary and secondary antibody), perform the immunostaining at this step. Wash thoroughly after antibody incubation.

B. Cell Staining

3. Add DRAQ5™ directly as supplied to a final concentration of 5 – 20μM to the cells. Alternatively, DRAQ5™ can be added to the fresh buffer/ media prior to resuspending the cells in it.

4. Gently mix the cells and then incubate for 5 – 30 minutes at Room temperature.

DRAQ5™ staining is accelerated at 37°C and incubation time may be reduced but this should be checked by titration and for each cell type.

Note: Protect cells from light during incubation period if other fluorescent stains (such secondary antibodies) have been applied to the cells, as they may otherwise suffer photo-bleaching.

5. Cells can be analyzed directly without further treatment or washing, preferably within two hours.

C. Data Analysis

DRAQ5™ can be used to discriminate nucleated from anucleated cells and to provide information on cell cycle status and ploidy in all type of cells.

It is important to consider the combinations of fluorochromes and filters for the experiments.

EXCITATION:

In flow cytometry, DRAQ5™ may be excited by wavelengths from 488 nm and up to 647 nm (Exλ_{max} 646 nm).

Despite low absorbance at 488 nm, this excitation offers optimal CVs for flow cytometry cell cycle analysis and convenient combination with FITC and R-PE conjugates and eGFP.

EMISSION:

Emission starts at 655 nm (Emλ_{max} 681 nm/ 697 nm dsDNA-bound).

Suitable filters include 695LP, 715LP or 780 LP.

For cell cycle analysis it is recommended to choose a filter (such as 715LP) which excludes a significant proportion of signal from the small fraction of unbound DRAQ5™.

Remove doublets and clumps by plotting DRAQ5™ peak height/ width versus DRAQ5™ peak area.

For cell cycle/ ploidy analysis, plot DRAQ5™ signals in linear mode.

6. FAQs

1. In which type of cells can I stain with DRAQ5™?

DRAQ5™ binds strongly to the A-T, A-T sites at the minor grove of DNA, which means that all nucleated cells will be stained by DRAQ5™.DRAQ5™ will not only stain all type of nucleated eukaryotic cells (primary or cell-line derived), but also bacterial cells (such as *E. coli* or *Bacillus*) and plant cells.

2. In which type of assays can I use DRAQ5™?

DRAQ5™ can be used in any assay where nuclear DNA staining is required.

Examples of these assays are:

- Live cell imaging:
  - Live cells or tissue (primary or cell-line derived)
  - Fixed cells (methanol, ethanol, paraformaldehyde, etc)
  - Permeabilized cells (by surfactant or naturally in apoptosis)
  - Tissue sections (frozen or paraffin-embedded)
  - Plant cells

- Fluorescence In Situ Hybridization (FISH)

- Flow Cytometry:
  - Cell cycle analysis
  - Cell ploidy/ aneuploidy
  - DNA content calculation

- HCS platforms:
  - Measurement of cell cycle position or ploidy of a cell/ population
  - Relative quantification of changes expression or turnover of a tagged protein

3. If I have more cells in my sample, do I need to add more DRAQ5™?

DRAQ5™ binds stoichiometrically to the DNA present in the cellular nuclei till it reaches an equilibrium point.

Our recommended standard concentrations of 5μM for cell microscopy and 5 – 20μM for flow cytometry should be sufficient to stain up to 1 x 10^6 cells/ml.

The quality of the sample will also influence the amount of DRAQ5™ needed; if the sample to analyze is very viscous (specifically for flow cytometry) we recommend using a final concentration 10 – 20μM.

4. Does DRAQ5™ stain mitochondria or RNA?

DRAQ5™ has high specificity for dsDNA and doesn't seem to bind to RNA. Although mitochondria contain dsDNA, no DRAQ5™ signals seem to be detected. This makes DRAQ5™ the ideal tool to study cell cycle profile, as all the signal will come from nuclear DNA.

5. Why shouldn't DRAQ5™-stained cells be washed?

There is no need to wash cells for imaging or flow cytometry. DRAQ5™ binds stoichiometrically to the DNA and reaches an equilibrium, which could be disrupted by long washes.

DRAQ5™ can be added at the final stage of the protocol prior to analysis, as it will be able to stain the nucleus very quickly (5 minutes incubation is enough for staining). If washes are essential in protocol, we recommend very brief washes.

6. Can I use DRAQ5™ with anti-fade mountants?

Yes. DRAQ5™ is perfectly compatible with anti-fade mountants such as Fluoroshield (ab104135), BrightMount (ab103746) or BrightMount Plus (ab103748). Just ensure that the mountant is the "native" product and does not contain DAPI as the DAPI quenches the DRAQ5™ staining signal.

7. If I use DRAQ5™ in live cells, can I do a long time-course experiment?

No. DRAQ5™ binds irreversibly to the cellular DNA which is ultimately cytotoxic to cells. DRAQ5™ staining occurs very quickly, so we suggest adding DRAQ5™ to live culture cells just before analysis. Short term experiments (< 2 hours) such as eGFP expression, mitochondrial tracking or calcium influx will not be affected by the dye toxicity.

For longer term viability assays, we recommend DRAQ7™ (ab109202).

8. Which fluorochromes can I use with DRAQ5™?

DRAQ5™ emits in far-red end of the spectra and therefore is compatible with fluorochromes with emission spectra lower than 600nm.

DRAQ5™ can be used with common labels such as eGFP/YFP, Cy2, FITC, Cy3, R-PE, and DyLight® 488, 549 and 594.

We do not recommend the use of DRAQ5™ with other far-red fluorochromes excited by the 488 ot 633 nm laser lines, such as PE-Cy7, PerCP-Cy5.5, APC, Texas Red or DyLight® 649.

9. How long can I keep my working dilution of DRAQ5™?

We recommend that if you dilute DRAQ5™, you use your working solution as soon as possible. Do not store diluted DRAQ5™; the agent is very stable but the dye could be lost from solution since it is formulated for immediate use.

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