IMMUNOLOGICAL SCIENCES

Product Data Sheet

# 0,5mM DRAQ5 Dye (Live & Fixed Cells)

**SPECTRAL CHARACTERISTICS:**
Ex$\lambda_{max}$ 600/646 nm  Em$\lambda_{max}$ 697 nm

DRAQ5™ is a novel far-red fluorescing DNA dye used for LIVE, permeabilized and fixed cells. It binds DNA with high affinity and stoichiometry. It can be used in combination with common visible range fluors, including FITC and R-PE and is compatible with common buffers. DRAQ5™ is highly compatible with existing protocols and a broad range of cytometry and microscopy instruments.

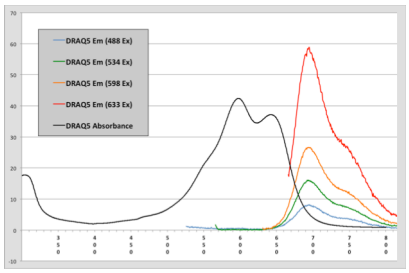

*Fig. 1. Spectral profile of DRAQ5™*

**APPLICATIONS:**

- Flow Cytometry – live (or fixed)
  Nucleated cell gating (no lyse, no wash), Cell cycle analysis
- Fluorescence Microscopy - live (or fixed)
- HCS & Cell-Based Assays
  Drug, RNAi, phenotypic screens, In-cell westerns

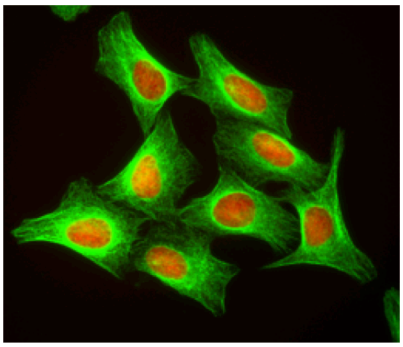
*Fig. 2. DRAQ5™ (red) counterstaining of fixed U2OS cells. AlexaFluor 488 antibody to β-tubulin (green).*

**NOTE:** As with any cell permeant DNA intercalating probe, DRAQ5™ may inhibit cell division in long term assays and should be tested for any effect.

**DETECTING DRAQ5™ SIGNALS:** (see Fig. 1)
**Flow cytometry:** DRAQ5™ can be excited by blue, green, yellow and red laser sources. Detect using longpass or bandpass filters above 660 nm and into the infra red e.g. 780/60 BP. For DNA content, select a filter above 700 nm. Exclude doublets and reduce acquisition rates.
**Microscopy / HCS Imaging Platform:** DRAQ5™ is optimally excited using yellow/red wavelengths. It is detected with far red filters above 660 nm. For DNA content, segment nuclear signals before measuring and detect above 700 nm, if possible.

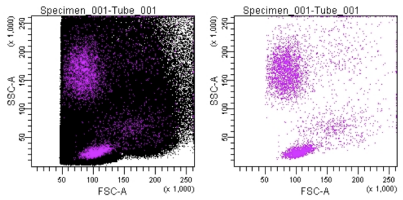

*Fig.3 No lyse, no wash gating of nucleated cells from whole bone marrow gating on DRAQ5™ signal.*

**PRESENTATION:** aqueous solution.
**STORAGE**: store at 2-8 °C. DO NOT FREEZE

| Catalogue n. | Description | Size |
|---|---|---|
| DR05500-50 | **Draq5** (5mM) | 50 ul |
| DR05500 | **Draq5** (0,5mM) | 500 ul |

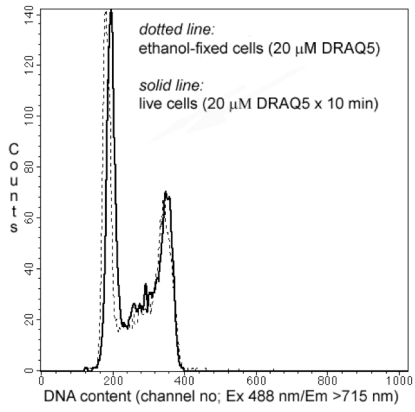

*Fig. 4. DNA content analysis in live or fixed cells*

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## Cell staining with DRAQ5 for DNA cell cycle analysis

# CELL STAINING WITH DRAQ5™ FOR DNA CELL CYCLE ANALYSIS BY FLOW CYTOMETRY OR BY CELL IMAGING

Reagents required:-

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

1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. Since no washing step is required, DRAQ5 will usually be the final staining procedure, after any cell treatment or labelling, prior to analysis.
3. Prepare cells for staining with DRAQ5™. Resuspend cells in appropriate buffer such as PBS at a concentration of ≤4 x $10^5$ / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section dimensions.
4. Add DRAQ5™ directly as supplied following the 10 µM or 20 µM pipetting volumes in table 1. (For simple flow cytometric gating of nucleated cells the concentration of DRAQ5™ may be reduced). This will be as an overlay for adherent cells / tissue sections, added to the chamber/well liquid directly or in fresh medium following a wash step.
5. Gently mix and then incubate for 5-30 minutes at room temperature.

nb: Protect from the light during this incubation period if other (immuno-) fluorescent stains have been applied to the cells, prior to the DRAQ5™labelling, and which may otherwise suffer photo-bleaching.
DRAQ5™ staining is accelerated at 37ºC and maybe reduced to 1-3 min. DRAQ5™ stains intact, live, fixed, permeabilized and dead cells.

6. Cells can be analysed directly without further treatment or washing.

It is also important to consider the combinations of fluorochromes and filters for the experiment:
**EXCITATION**: DRAQ5™ may be excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Ex$\lambda_{max}$ 646 nm). Despite low absorbance at 488 nm this excitation may offer optimal CVs for flow cytometric cell cycle analysis whilst allowing convenient combination with FITC and R-PE conjugates and EGFP.
**EMISSION**: this starts at 665 nm (Em$\lambda_{max}$ 681 nm / 697 nm intercalated to dsDNA). Suitable filters include 695LP, 715LP or 780 LP. For cell cycle analysis it is recommended to choose a filter (such as 715 LP) which excludes a significant proportion of signal from the small fraction of unbound DRAQ5™.

**Table 1:**
Ready reckoner for volumes of DRAQ5™ (5mM) required for various cell concentrations:-

| Cell sample preparation: | | VOLUME OF DRAQ5™ (AS SUPPLIED) REQUIRED FOR A CONCENTRATION OF: | | |
|---|---|---|---|---|
| **No. of cells:** | **in volume:** | **5 µM** | **10 µM** | **20 µM** |
| 1 x $10^6$ | 2500 µl | 2.5 µl | 5 µl | 10 µl |
| 4 x $10^5$ | 1000 µl | 1 µl | 2 µl | 4 µl |
| 2 x $10^5$ | 500 µl | 0.5 µl | 1 µl | 2 µl |
| 1 x $10^5$ | 250 µl | 0.25 µl | 0.5 µl | 1 µl |
| 5 x $10^4$ | 125 µl | 0.13 µl | 0.25 µl | 0.5 µl |

DRAQ5™ is intended for research purposes only.

## LIVE cell staining with DRAQ5™ for nuclear visualisation

## LIVE CELL STAINING WITH DRAQ5™ FOR NUCLEAR VISUALIZATION BY HCS IMAGING PLATFORM OR LASER SCANNING CONFOCAL / EPIFLUORESCENCE MICROSCOPY

Reagents required:-

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

1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. DRAQ5™ is usually added as the last stain in a labelling procedure since no washing is required or conveniently in assay medium for a live cell assay.
3. Prepare cells for staining with DRAQ5™. Resuspend cells in appropriate buffer such as PBS at a concentration of ≤4 x $10^5$ / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section dimensions.
4. Add DRAQ5™ directly as supplied following the 5 µM or 10 µM pipetting volumes in table 1. This will be as an overlay for adherent cells / tissue sections, added to the chamber/well liquid directly or in fresh medium following a wash step.
5. Gently mix and then incubate for 5-30 minutes at room temperature. 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 hr.) at 1 µM prior to any agonist / antagonist addition.
   nb: Protect from the light during this incubation period if other (immuno-) fluorescent stains have been applied to the cells, prior to the DRAQ5™ labelling, and which may otherwise suffer photo- bleaching.
   DRAQ5™ staining is accelerated at 37ºC and maybe reduced to 1-3 min. DRAQ5™ stains intact, live, permeabilized and dead cells.
6. Cells can be analysed directly without further treatment or washing.

It is also important to consider the combinations of fluorochromes and filters for the experiment:

**EXCITATION**: DRAQ5™ may be sub-optimally excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Ex$\lambda_{max}$ 646 nm). Typically, for cell imaging, excitation is typically performed with either 561 nm, 633 nm or 647 nm wavelengths.

**EMISSION**: Em$\lambda_{max}$ 681 nm / 697 nm intercalated to dsDNA. Suitable filters include 695LP, 715LP or 780 LP. DRAQ5™ has no spectral emission overlap with FITC, R-PE, GFP and many other fluorescing proteins allowing image acquisition in one scan.

**Table 1:**

Ready reckoner for volumes of DRAQ5™ (5mM) required for various cell concentrations:-

| **Cell sample preparation:** | | **VOLUME OF DRAQ5™ (AS SUPPLIED) REQUIRED FOR A CONCENTRATION OF:** | | |
|---|---|---|---|---|
| **No. of cells:** | **in volume:** | **5 µM** | **10 µM** | **20 µM** |
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| 1 x $10^5$ | 250 µl | 0.25 µl | 0.5 µl | 1 µl |
| 5 x $10^4$ | 125 µl | 0.13 µl | 0.25 µl | 0.5 µl |

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## Fixed cell/tissue staining with DRAQ5 for nuclear visualization

**FIXED CELL / TISSUE STAINING WITH DRAQ5™ FOR NUCLEAR VISUALIZATION BY HCS IMAGING PLATFORM OR LASER SCANNING CONFOCAL / EPIFLUORESCENCE MICROSCOPY**

Reagents required:-

O DRAQ5 ; Phosphate Buffered Saline (PBS, without sodium azide) or other culture medium ; Paraformaldehyde

A. **SEPARATE FIXATION AND COUNTERSTAINING STEPS** (typically for cells / tissues where one or more external (immuno-) fluorescent stains will be applied):

1. Read the supplied Material Safety Data Sheet before handling DRAQ5
2. DRAQ5 is usually added last in a procedure as no washing is required.
3. Prepare the sample for fixation and subsequent staining with DRAQ5.
4. Prepare separate working solutions of 4% formaldehyde and 5 µM DRAQ5 in PBS, pipetting 1 µl of DRAQ5, as supplied, into 1000 µl of PBS.
5. Overlay the slide or chamber/well with the 4% formaldehyde solution. Incubate for 15-30 minutes at room temperature / 37ºC.
6. Gently aspirate the formaldehyde solution, and wash with PBS.
7. Perform any necessary permeabilization, (immuno-)staining and blocking steps.
8. Overlay the washed, aspirated sample with 5µM DRAQ5 solution. Incubate for 15-30 minutes at room temperature / 37ºC, in the dark.

NOTE: Protect from the light during these incubation periods if other (immuno-) fluorescent stains have been applied to the samples, which may otherwise suffer photo-bleaching.Samples can be analysed directly without further treatment or washing. DRAQ5 staining is accelerated at 37ºC and incubation time may be reduced but this should be checked by titration and for each cell type. DRAQ5 stains all nucleated cells.

It is also important to consider the combinations of fluorochromes and filters:

**EXCITATION**: DRAQ5™ may be sub-optimally excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm ($Ex\lambda_{max}$ 646 nm). Typically, for cell imaging, excitation is typically performed with either 561 nm, 633 nm or 647 nm wavelengths.

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B. **FOR A COMBINED FIXATION AND COUNTER-STAINING STEP** (typically for cells / tissues expressing an endogenous fluorescent protein as the only analyte e.g. translocation of a GFP-tagged transcription factor), simplifying the protocol:

1. Read the supplied Material Safety Data Sheet before handling DRAQ5™
2. DRAQ5™ is usually added last in a labeling as washing is not required.
3. Prepare the sample for fixation and subsequent staining with DRAQ5™.
4. Prepare separate working solutions of 8% formaldehyde (FA)and 10 µM DRAQ5 in PBS, pipetting 2 µl of DRAQ5, as supplied, into 1000 ul of PBS.
5. Overlay the slide or chamber/well with equal volumes of formaldehyde and DRAQ5 solutions. Alternatively, prepare a pre-mix of the DRAQ5 and FA working solutions to simplify and speed the workflow for multiple samples. Overlay the cells with this mixture. Incubate for 15-30 minutes at room temperature / 37ºC, in the dark.

   NOTE: Protect from light during the incubation period if other (immuno-) fluorescent stains are present, which may otherwise suffer photo-bleaching.
6. Samples can be analysed directly without further treatment or washing.

DRAQ5™ staining is accelerated at 37ºC and incubation time may be reduced but this should be checked by titration and for each cell type. DRAQ5™ stains all nucleated cells.

It is important to consider combinations of fluorochromes / filters for the experiment:

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