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

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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 (Exλ_{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 (Emλ_{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™.

LIVE CELL STAINING WITH DRAQ5™ FOR NUCLEAR VISUALIZATION BY IMAGER OR LASER SCANNING CONFOCAL / EPIFLUORESCENCE MICROSCOPY

Reagents required:-

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

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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 (Exλ_{max} 646 nm). Typically, for cell imaging, excitation is performed with either 633 nm or 647 nm wavelengths to achieve.

EMISSION: this starts at 665 nm (Emλ_{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**
1×10^6	2500 µl	2.5 µl	5 µl	10 µl
4×10^5	1000 µl	1 µl	2 µl	4 µl
2×10^5	500 µl	0.5 µl	1 µl	2 µl
1×10^5	250 µl	0.25 µl	0.5 µl	1 µl
5×10^4	125 µl	0.13 µl	0.25 µl	0.5 µl

DRAQ5™ is intended for research purposes only.