EXAMPLE PROTOCOLS FOR DRAQ7™

CELL STAINING WITH DRAQ7™ FOR DEAD CELL / APOPTOSIS EVALUATION BY FLOW CYTOMETRY

Reagents required:-

- DRAQ7™
- Phosphate Buffered Saline (PBS, without sodium azide) or other culture medium

1. Read the supplied Material Safety Data Sheet before handling DRAQ7™
2. Since no washing step is required, DRAQ7™ will usually be the final staining procedure, after any cell treatment or labeling, prior to analysis.
3. If your protocol requires surface antibodies or apoptosis indicators such as Annexin V –FITC or JC-1, perform these steps first.
4. Prepare cells for staining with DRAQ7™. Resuspend cells in appropriate buffer such as PBS at a concentration of ≤5 x 10^5 / ml in a test tube. For adherent cells estimate the number of cells based on confluence level, tissue section dimensions or directly by cell counting.
5. DRAQ7™ is supplied ready-to-use. For each 0.5 ml of cell suspension add 5 µl of DRAQ7™ (to give an optimised final concentration of 3 µM).
6. Gently mix by pipetting and then incubate for 10 minutes at 37°C / room temperature, in the dark.

   NOTE: Protect from the light during this incubation period if other (immuno-) fluorescent stains have been applied to the cells, which may otherwise suffer photo-bleaching.

   DRAQ7™ staining is accelerated at 37ºC and incubation time may be reduced but this should be checked by titration and for each cell type.

   DRAQ7™ stains apoptotic (membrane-compromised), fixed, permeabilized and dead/dying cells.

7. 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: DRAQ7™ may be excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Exλ_{max} 599/644 nm). Despite low absorbance at 488 nm this single excitation source allows convenient combination with FITC and R-PE conjugates and EGFP. **EMISSION**: this starts at 665 nm (Emλ_{max} 678 nm / 694 nm intercalated to dsDNA). Suitable filters include 695LP, 715LP or 780 LP.

USE OF DRAQ7™ IN REAL-TIME, DYNAMIC CELL VIABILITY ASSAYS

DRAQ7™ has been shown not to have any effect on the proliferation rate of cells in long-term culture assays (example: SU-DHL-4 cells cultured for 96 hours, continuously exposed to 10 µM DRAQ7™). DRAQ7 can then be used to report, in real-time, the cytotoxic or apoptotic effect of a specific treatment on cells e.g. pharmacological agent, RNAi, virus, antibody-dependent complement-mediated killing, in vitro *toxicology testing.*

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1. Read the supplied Material Safety Data Sheet before handling DRAQ7™.
2. Add DRAQ7™ directly to the cell culture medium: 10 µl, as supplied, per 1.0 ml of culture medium, mix for a final concentration of 3 µM.
3. Remove aliquots as required and analyse for far-red (> 665 nm) fluorescing cells relative to controls by flow cytometry or microscopy. Normally cells will not need to be washed prior to analysis.

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

EXCITATION: DRAQ7™ may be sub-optimally excited by wavelengths from 488 nm (in flow cytometry) and up to 647 nm (Exλ_{max} 599/646 nm). Typically, for cell imaging, excitation is performed with either 633 nm or 647 nm wavelengths.

EMISSION: this starts at 665 nm (Emλ_{max} 678 nm / 694 nm intercalated to dsDNA). Suitable filters include 695LP, 715LP or 780 LP. DRAQ7™ has no spectral emission overlap with FITC, R-PE, GFP and many other fluorescing proteins allowing image acquisition in one scan.

ANALYSIS BY FLOW CYTOMETRY: what you should expect to see

To establish the position of DRAQ7$^+$ cells a control experiment may be performed. Analyze untreated, unstained control cells, plotting the results as an intensity histogram, exciting/detecting in all available channels to determine the negative event distribution and any biofluorescence. Analyze treated, unstained cells similarly. Based on both samples, adjust the instrument settings to place the negative population in the first log decade.

Add DRAQ7 to a new aliquot of untreated control cells according to the protocol. Split the aliquot in half, analyzing one half to establish the position of the DRAQ7$^-$ cells.

Permeabilize the remaining half of this aliquot of untreated cells with the addition of 1% Triton-X100, vortex to mix and analyze to establish the position of DRAQ7$^+$ (i.e. membrane-compromised) cells. Alternatively, add DRAQ5™ (at 20 µM) and incubate for 10 minutes at 37°C. The position of DRAQ5$^+$ events is the upper limit for the DRAQ7$^+$ event signal.

These control experiments should allow setting of DRAQ7$^-$ / DRAQ7$^+$ gates.

FIXED CELL/TISSUE STAINING WITH DRAQ7™ FOR NUCLEAR VISUALIZATION BY HCS IMAGING PLATFORM OR LASER SCANNING CONFOCAL / EPIFLUORESCENCE MICROSCOPY

Reagents required:-

- DRAQ7™
- 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 DRAQ7™
2. DRAQ7™ is usually added last in a procedure as no washing is required.
3. Prepare the sample for fixation and subsequent staining with DRAQ7™.
4. Prepare separate working solutions of 4% formaldehyde and 5 µM DRAQ7 in PBS, pipetting 17 µl of DRAQ7, 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 DRAQ7 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.

9. Samples can be analysed directly without further treatment or washing.

DRAQ7™ staining is accelerated at 37ºC and incubation time may be reduced but this should be checked by titration and for each cell type. DRAQ7™ stains apoptotic (membrane-compromised) fixed, permeabilized and dead/dying cells.

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

EXCITATION: For cell imaging, DRAQ7™ (Exλ_{max} 599/646 nm) excitation is performed with either 633 nm or 647 nm wavelengths.

EMISSION: this starts at 665 nm (Emλ_{max} 678 nm / 694 nm intercalated to dsDNA). Suitable filters include 695LP, 715LP or 780 LP. DRAQ7™ has no spectral emission overlap with FITC, R-PE, GFP and many other fluorescing proteins allowing image acquisition in one scan.

B. **FOR A COMBINED FIXATION AND COUNTERSTAINING 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 DRAQ7™
2. DRAQ7™ is usually added last in a labeling as washing is not required.
3. Prepare the sample for fixation and subsequent staining with DRAQ7™.
4. Prepare separate working solutions of 8% formaldehyde and 10 µM DRAQ7 in PBS, pipetting 34 µl of DRAQ7, as supplied, into 1000 ul of PBS.
5. Overlay the slide or chamber/well with equal volumes of formaldehyde and DRAQ7 solutions. Alternatively, overlay a pre-mix of the two working solutions to simplify and speed the workflow for multiple samples. 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.

DRAQ7™ staining is accelerated at 37ºC and incubation time may be reduced but this should be checked by titration and for each cell type. DRAQ7™ stains apoptotic (membrane-compromised) fixed, permeabilized and dead/dying cells.

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EMISSION: this starts at 665 nm (Emλ_{max} 678 nm / 694 nm intercalated to dsDNA). Suitable filters include 695LP, 715LP or 780 LP. DRAQ7™ 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 DRAQ7™ (0.3mM) required for various cell concentrations:-

Cell sample preparation:		VOLUME OF DRAQ7™ (AS SUPPLIED) REQUIRED FOR A CONCENTRATION OF:		
No. of cells:	**in volume:**	**3 µM**	**5 µM**	**10 µM**
5 x 10^5	1000 µl	10 µl	17 µl	34 µl
2.5 x 10^5	500 µl	5 µl	8.5 µl	17 µl
1 x 10^5	200 µl	2 µl	3.4 µl	6.8 µl
5 x 10^4	100 µl	1 µl	1.7 µl	3.4 µl

DRAQ7™ is intended for research purposes only.

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