



PRODUCT: DRAQ7 DROP & GO™
PRODUCT CODES: DR72524, DR77524

PRESENTATION: blue aqueous solution
STORAGE: store at 15-30°C. DO NOT FREEZE

DESCRIPTION:

DRAQ7™ is a novel far-red fluorescing viability dye that ONLY stains nuclei in DEAD or membrane-compromised cells. It easily combines with common fluors, e.g. FITC, R-PE and is compatible with common buffers. DRAQ7™ has applications in imaging, cytometry and screening, in existing protocols across most platforms. Uniquely DRAQ7™ can be used in long-term cell health monitoring for many days.

DRAQ7 DROP & GO™ is a convenient ready-to-use formulation of DRAQ7™ in a dropper bottle. This product is designed for use in the flow cytometry or cell culture sample preparation area.

APPLICATIONS:

- Flow Cytometry – dead/apoptotic cell enumeration and exclusion
- Cell Sorting – dead cell gating (to “dump” channel) e.g. single cell transcriptomics
- Fluorescence Microscopy – cell health assays

BEFORE STARTING:

Read the [SDS](#). Wear protective clothing, safety goggles and laboratory gloves.

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:

PBS (azide-free), culture medium (CM), CM without phenol red[‡] (“Imaging CM”), paraformaldehyde, Triton-X 100, antibodies.

DETECTING DRAQ7™ SIGNALS: (see Fig. 1)

Flow cytometry: DRAQ7™ can be excited by blue, green, yellow, red light. Detect using longpass or bandpass filters above 660 nm into the infra-red e.g. 780/60 BP.

Microscopy / HCS Imaging Platform: DRAQ7™ is optimally excited using yellow / red wavelengths. It is detected with far-red filters above 660 nm e.g. “Cy5”.

DRAQ7™ has no spectral overlap with FITC, GFP, PE, YFP, PerCP or rhodamine-based chromophores and requires only limited compensation with APC, PE-Cy5 and PE-Cy7.

EXAMPLE PROTOCOLS

PROTOCOL 1:

CELL STAINING FOR DEAD / APOPTOTIC CELL EVALUATION BY FLOW CYTOMETRY

1. After all other procedures are completed, prepare cells for staining with DRAQ7™: resuspend cells in appropriate buffer (e.g. PBS) at a concentration of $\leq 5 \times 10^5$ / ml in a test tube.
2. For each 0.5 ml of cell suspension add 1-2 drops of DRAQ7 DROP & GO™.
3. Gently mix by pipetting. Incubate for 10 min. at 37 °C, in the dark.
4. Analyze without further treatment or washing.

NOTE: Protect samples from light during incubations, particularly if other (immuno-) fluorescent stains have been applied, which may otherwise suffer photo-bleaching.

SPECTRAL CHARACTERISTICS:

Ex λ_{\max} 599/644 nm Em λ_{\max} 694 nm

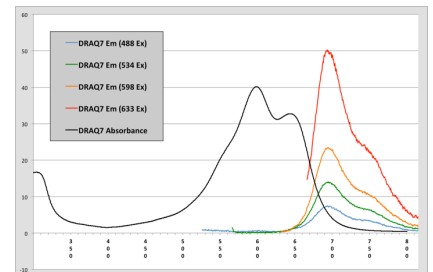


Figure 1. Spectral properties of DRAQ7™ - spectral compatibility with UV-excited and most vis. range fluorochromes for multi-colour analysis. Detection from blue excitation is achievable only by flow cytometry.

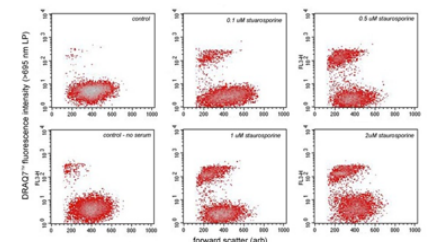


Figure 2. Lymphoma cells treated with increasing quantities of staurosporine (STS). DRAQ7™ reports STS-induced apoptosis and cell death in dose-dependent manner with clear separation of positive and negative events.

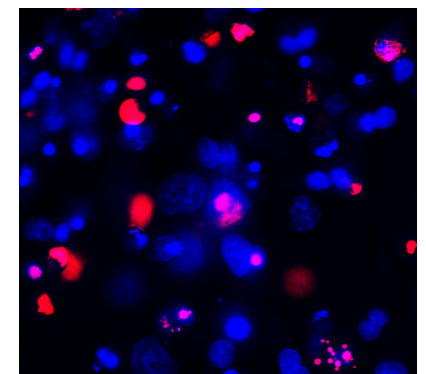


Figure 3. A549 cells treated with Gemcitabine and subsequently labelled with DRAQ7™ (red, dead cell nuclei) and Hoechst 33342 (blue, all cell nuclei). Data courtesy of Dr Gareth Griffiths, Imagen Biotech.

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PROTOCOL 2:

MONITORING CELL VIABILITY IN LIVE END-POINT & REAL-TIME, DYNAMIC CELL-BASED ASSAYS

Uniquely, as a viability dye, DRAQ7™ can be used in long-term assays and has been shown not to have any impact on living cells over several days of exposure (Akagi, et al., 2013). DRAQ7 DROP & GO™ makes addition of DRAQ7™ to culture medium very simple. However, for very large experiments we recommend DR71000 or DR710hc. Contact us for guidance on this.

1. For each 2 ml aliquot of Imaging CM‡ add 3* drops of DRAQ7 DROP & GO™.
2. For flow cytometric monitoring remove aliquots as required, adding end-point stains as needed.
3. Analyse for far-red (> 665 nm) events relative to controls, by flow cytometry or microscopy. No washing is required.

* It may be necessary to assess the correct dose of DRAQ7 DROP & GO™ for your cell culture assay (due to cell density, microtissue dimensions, tissue thickness, etc.). Other functional probes are typically added to culture medium at this point (e.g. TMRM). The final concentration of DRAQ7™ is 1.3 µM, and widely applied by users in published examples. This is a suitable concentration of DRAQ7™ when it is paired with the metabolic cell health probe Calcein AM.

‡ Prior to the start of time-lapse fluorescence imaging, if the initial culture medium (CM) contains phenol red indicator it should be replaced by its equivalent without phenol red (i.e. "Imaging CM") when adding DRAQ7™ into the assay. This avoids unwanted background fluorescence.

ANALYSIS BY FLOW CYTOMETRY

What you should expect to see:

Run controls to set DRAQ7+ event position: analyse i) untreated, unstained cells and ii) treated, unstained cells, plotting results as an intensity histogram, exciting/detecting in all available channels to determine the negative event distribution and biofluorescence. Adjust settings to place the negative population in the first log decade. iii) Add DRAQ7™ to a new aliquot of untreated control cells according to the protocol. Split the aliquot in two, analysing one half to establish the position of the DRAQ7- cells. With the remaining half of this aliquot add 1% Triton-X100, vortex, incubate for 10 minutes. Analyse to establish the position of DRAQ7+ cells setting the upper limit for the DRAQ7+ event signal. These control experiments should allow setting of DRAQ7- / DRAQ7+ gates.

"Dual-Beam Exclusion" of DRAQ7+ events

A unique feature of DRAQ7™ is that it is multi-line excitable. With appropriate selection of the other fluorophores, on a multi-laser instrument this allows definition of a unique population when one plots two separate fluorescence emission channels generated by two separate lasers against each other. Thus, DRAQ7+ events can be discretely excluded from all other channels with a "DRAQ7-not" gate either during or post-acquisition. (Blanco & Edward, 2018).

Key references:

Akagi, J. et al. (2013) Cytom Part A 83A: 227-234
Edward, R. (2018) in *High Content Screening* (pp. 3-19)
Humana Press, New York.

Blanco, A. & Edward R. Poster presentation, CYTO 2018, Prague
Smith, P.J. et al. (2013) Cytom Part A 83A: 161-169

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BioStatus products are the subject of several international patents. DRAQ7™, DRAQ7 DROP & GO™ and DRAQ5™ are trademarks of BioStatus Limited.

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