DRAQ7™ TECHNICAL DATA SHEET

PRODUCT: DRAQ7™
PRODUCT CODES: DR70250; DR71000; DR710HC

PRESENTATION: blue aqueous solution.
STORAGE: store at 2-8 °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.

APPLICATIONS:
- Flow Cytometry – dead/apoptotic cell enumeration and exclusion
- Cell Sorting – dead cell gating (to "dump" channel)
- Fluorescence Microscopy – cell health assays
- HCS – cell health and *in vitro* toxicology

BEFORE STARTING:
Read the SDS. Wear protective clothing, safety goggles and laboratory gloves. Check the concentration of DRAQ7™ stated on the vial label.

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:
Phosphate-Buffered Saline (PBS, without azide), culture medium, DRAQ5™, paraformaldehyde, Triton-X 100, antibodies, plasticware, slides/coverslips.

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.

EXAMPLE PROTOCOLS

PROTOCOL 1:
CELL STAINING FOR DEAD / APOPTOTIC CELL EVALUATION BY FLOW CYTOMETRY
As no washing is required, DRAQ7™ is usually added last, prior to analysis. If using surface antibodies, Hoechst 33342 for SP analysis (see Smith. et al., 2013) or apoptosis indicators e.g. Annexin V–FITC or JC-1, perform these procedures first.

1. Prepare cells for staining with DRAQ7™: resuspend cells in appropriate buffer (e.g. PBS) at a concentration of ≤5 x 10^5 / ml in a test tube.
2. DRAQ7™ is supplied ready-to-use. For each 0.5 ml of cell suspension add 5 µl of DRAQ7™ (for an optimised final concentration of 3 µM).
3. Gently mix by pipetting. Incubate for 10 min. at 37 °C / room temp, in the dark.
4. Analyze without further treatment / washing.

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

DRAQ7™ staining is accelerated at 37 °C and incubation time may be reduced but this should be checked by titration and for each cell type. DRAQ7™ stains membrane-compromised (e.g. apoptotic), fixed, permeabilised and dead cells.

*Use a volume of 100-200 µl/coverslip; 100 ul/96-MTP, 30 ul/384-MTP, 10 ul/1536-MTP well.

SPECTRAL CHARACTERISTICS:
Exλ_{max} 599/644 nm Emλ_{max} 694 nm

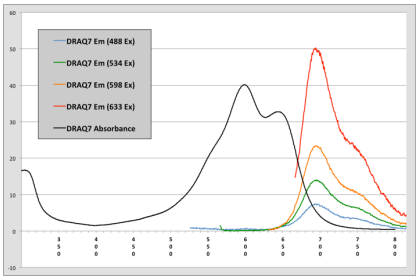

Figure 1. Spectral properties of DRAQ7™ - spectral compatibility with UV-excited and most vis. range fluorochromes for multi-colour analysis. Detection from blue excitation is achievable only by flow cytometry.

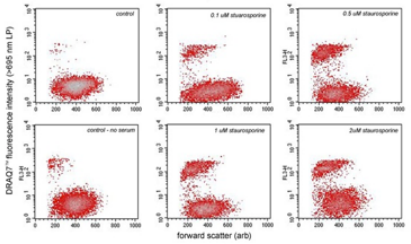

Figure 2. Lymphoma cells treated with increasing quantities of staurosporine (STS). DRAQ7™ reports STS-induced apoptosis and cell death in dose-dependent manner with clear separation of positive and negative events.

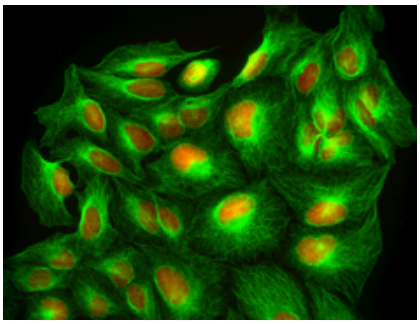

Figure 3. Formaldehyde-fixed U2OS cells labelled with DRAQ7™ (red, nuclei) and AlexaFluor 488 antibody to ß-tubulin (green).

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PROTOCOL 2:

MONITORING CELL VIABILITY IN 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).

1. Add DRAQ7™ at any stage of the assay directly to cells in culture medium: 10 µl, as supplied, per 1.0 ml of culture medium, and mix for a final concentration of 3 µM*. Add other real-time functional probes at this point (e.g. TMRM).
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.

PROTOCOL 3:

FIXED CELL COUNTERSTAINING FOR HCS IMAGING PLATFORM OR FLUORESCENCE MICROSCOPY

DRAQ7™ can be used as a fluorescent counterstain to image fixed cells, similar to DRAQ5™

A. SEPARATE FIXATIVE & COUNTERSTAIN STEPS

1. Prepare separate working solutions of 4% formaldehyde (FA) and 5 µM DRAQ7™ in PBS.
2. Overlay the slide or well with 4% FA. Incubate for 15-30 minutes at room temperature / 37 °C.
3. Gently aspirate FA, and wash with PBS.
4. Perform any necessary permeabilization, (immuno-)staining and blocking steps.
5. Wash with PBS to remove any residual Triton X-100, if used, and aspirate the sample. Overlay with DRAQ7™*.
6. Incubate for 10-20 minutes at room temperature. n.b. protect from light.
7. Analyze without further treatment / washing. False colour DRAQ7™ images in red.

B. COMBINED FIXATIVE & COUNTERSTAIN

1. Prepare separate working solutions of 8% formaldehyde (FA) and 10 µM DRAQ7™ in PBS.
2. Overlay the slide or well with equal 0.5 volumes* of formaldehyde and DRAQ7™ solutions.
   Alternatively, make a pre-mix of DRAQ7™ and FA working solutions to overlay 1 volume per sample*.
3. Incubate for 10-20 minutes at room temperature. n.b. protect from light.
4. Analyze without further treatment / washing. False colour DRAQ7™ images in red.

ANALYSIS BY FLOW CYTOMETRY

What you should expect to see:

Run controls to set the position of DRAQ7$^+$ events: analyse i) untreated, unstained control 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 instrument 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 either a) add DRAQ5™ (at 20 µM) and incubate for 10 minutes at 37°C or b) add 1% Triton-X100, vortex. 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. (See separate application note for more details).

Key references:

Akagi, J. et al. (2013) Cytometry Part A 83A: 227-234
Smith, P.J. et al. (2013) Cytometry Part A 83A: 161-169
Edward, R. (2012) Meth Enzymol: 505: 23-45

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