DRAQ7™ TECHNICAL DATA SHEET

PRODUCT: DRAQ7™
PRODUCT CODES: DR70250; DR71000; DR71005; 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) e.g. for transcriptomics
- Fluorescence Microscopy – time-lapse 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:
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.

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, after surface antibodies, Hoechst 33342 (e.g. for SP analysis; see Smith. et al., 2013), metabolic (e.g. Calcein AM§) or apoptosis indicators (e.g. Annexin V–FITC, JC-1).*

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 a final concentration of 3 µM§, or as optimised.
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 µl/96-MTP, 30 µl/384-MTP, 10 µl/1536-MTP well.

§ When combined with Calcein AM, keep DRAQ7™ below 2.0 µM.

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

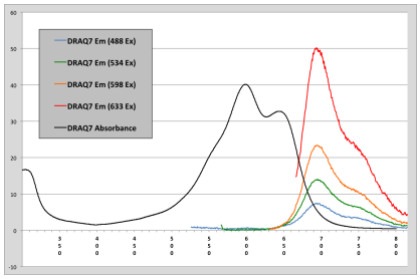

Figure 1. Spectral properties of DRAQ7™ - spectral compatibility with UV-excited and most vis. range fluorochromes for multi-colour analysis. Detection from blue excitation is achievable only by flow cytometry.

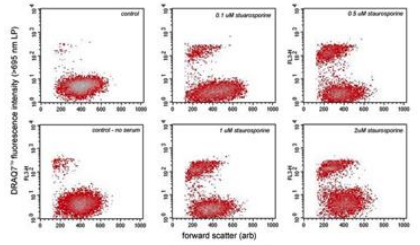

Figure 2. Lymphoma cells treated with increasing quantities of staurosporine (STS). DRAQ7™ reports STS-induced apoptosis and cell death in dose-dependent manner with clear separation of positive and negative events.

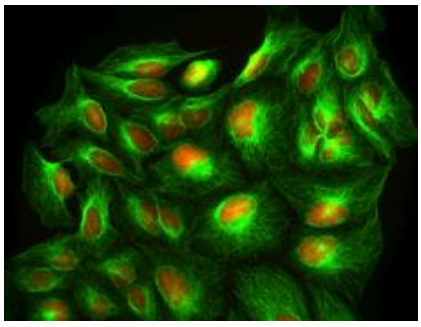

Figure 3. Formaldehyde-fixed U2OS cells labelled with DRAQ7™ (red, nuclei) and AlexaFluor 488 antibody to β-tubulin (green).

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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 (CM): 3.3 – 5.0 µl, as supplied, per 1.0 ml of CM‡, and mix: final concentration 1.0 – 1.5 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.

‡ 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.

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, wash with PBS. Perform any permeabilization, (immuno-)staining and blocking steps.
4. Wash with PBS to remove any residual Triton X-100, if used, and aspirate the sample. Overlay with DRAQ7™*.
5. Incubate for 10-20 minutes at room temperature. n.b. protect from light.
6. 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 and overlay 1 volume per sample*.
3. Follow steps 5 & 6 from Protocol 3A.

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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FOR FURTHER INFORMATION PLEASE CONTACT:
BioStatus Limited

Website:	www.biostatus.com
Technical Support:	support@biostatus.com
Re-ordering:	enquiry@biostatus.com
Telephone:	+44 1509 558163

BioStatus Limited
56A Charnwood Road, Shepshed, Leicestershire LE12 9NP UK
Company registered in England and Wales. No. 3079239

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