BIOSTATUS
DRAQ9™ TECHNICAL DATA SHEET

PRODUCT: DRAQ9™
PRODUCT CODES: DR90200; DR91000
PRESENTATION: blue DMSO solution
STORAGE: store at -20 °C; use above +20 °C

DESCRIPTION

DRAQ9™ is a novel far-red fluorescing cell permeant probe that labels membranous and vesicular structures in cytoplasm. It can be combined with common UV-excited and visible-range fluors, including GFP, and is compatible with common cell culture media and buffers. DRAQ9™ enables long-term cell tracking and cell painting for high content phenotypic screening. DRAQ9™ does not label the cell nucleus.

APPLICATIONS

- Cell painting / Cell mosaic: for non- *a priori* screening of cell changes on treatment
- Long-term cell tracking – non-toxic, stable labeling of cells over several days
- Longitudinal labeling of spheroids – correlation with cellular mass

Fluorescence microscopy & High content screening platforms.

BEFORE STARTING

Read the SDS. Wear protective clothing, safety goggles and laboratory gloves. Check the concentration of DRAQ9™ stated on the vial label.

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED

PBS (azide-free), culture medium (CM), CM without phenol red ("Imaging CM"), paraformaldehyde, warming bath.

DETECTING DRAQ9™ SIGNALS (see Fig. 1)

DRAQ9™ is optimally excited using yellow/red wavelengths. It is detected with far-red filters above 660 nm, using a broad band-pass or long band-pass filter, for example: Chroma Part No. 49019 (Ex. 620/20nm, Em. 665LP, HQ 700/75)

PREPARING DRAQ9™ FOR USE

DRAQ9™ is supplied at 1 mM in DMSO. As the melting point of DMSO is 19°C it is necessary to warm the product vial in a warming bath to above 19°C before pipetting the required amount of DRAQ9™.

Dilute the required amount of DRAQ9™ with PBS or culture medium to a working stock solution of 20 µM (i.e. 1:50), before returning the vial to its outer box and storing in the -20°C freezer.

Notes:
If procedures demand it, make up diluted (i.e. working conc$^{n.}$) DRAQ9™ required for up to one day's lab work e.g. total volume required set up a series of wells.

Freeze-thaw cycles do not affect the quality or performance of DRAQ9™, therefore aliquoting is not necessary.

SPECTRAL CHARACTERISTICS:

Exλ_{max} 605/655 nm Emλ_{max} 698 nm

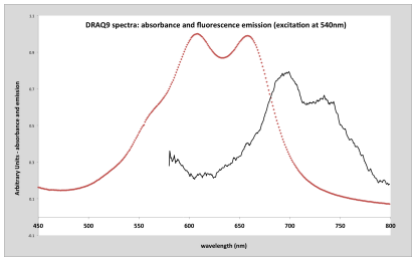

Fig. 1. Spectral profile of DRAQ9™

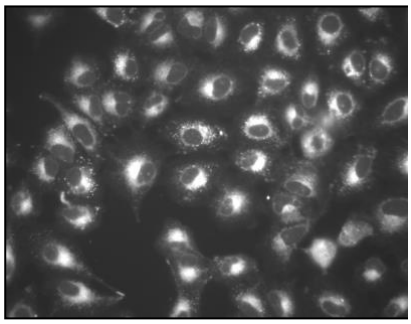

Fig. 2. DRAQ9™ labelling of live U2OS cells for 48 h at 2 µM - showing a number of cells in mitosis.

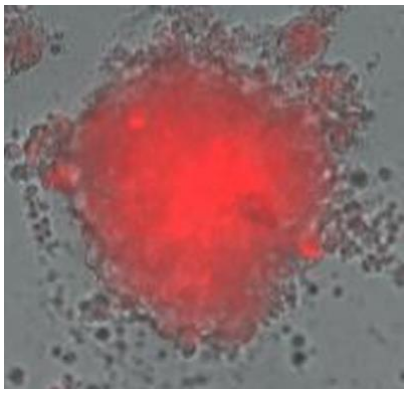

Fig.3. 6-day U2OS spheroid with continuous labelling with DRAQ9™ at 2µM

BioStatus Limited
56A Charnwood Road, Shepshed, Leicestershire LE12 9NP, United Kingdom
T +44 1509 558 163 | E enquiry@biostatus.com | W www.biostatus.com

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EXAMPLE PROTOCOLS

The indicator phenol red may introduce unwanted background in live cell imaging of DRAQ9™ and other red/far-red fluorescing reporters and any CM containing it should be replaced with a phenol red-free version of the CM ("Imaging CM") prior to the start of the period of time-lapse or live-cell endpoint imaging.

PROTOCOL 1:

LONG-TERM LIVE CELL TRACKING

1. If cells are already in culture medium simply pipette 11% v/v of the 10X working solution of DRAQ9™ into the volume of CM in the well or chamber.
   Or, preferably..
   Add 1 volume of DRAQ9™ working stock solution to 9 volumes of CM, mix and overlay adherent cells or use to re-suspend a cell pellet prior to dispensing into wells. The final concentration of DRAQ9™ should be 2 µM.
   Note: at this point, other real-time cell health probes can be added; for example, DRAQ7™ (for viability), TMRM for mitochondrial membrane potential.
2. After an initial incubation period of 20-30 minutes, cells can then be imaged repeatedly to follow their response to treatments over several days.
   Note: DRAQ9™ is <u>not</u> intended as a dilution dye and therefore daughter cells will have the same intensity as the parental cells and tracked as unique objects. On replacing CM, fresh CM should contain newly-prepared DRAQ9™ at 2µM.

PROTOCOL 2:

FIXATION OF CELLS FOLLOWING LONGITUDINAL LABELING WITH DRAQ9™

1. Fix cells by overlaying with a 4% solution of formaldehyde in PBS. Incubate for 30 minutes at RT / 37°C.
2. Aspirate off formaldehyde. Wash cells with PBS.
3. Aspirate and apply mountant* and coverslip to slides or tamp excess liquid from microtiter plate wells prior to imaging.

PROTOCOL 3:

CELL STAINING FOR CELL "PAINTING" / CELL "MOSAIC" STAINING IN PHENOTYPIC SCREENING

1. Fix cells by overlaying with a 4% solution of formaldehyde in PBS. Incubate for 30 minutes at RT / 37°C.
2. Aspirate off formaldehyde. Wash cells with PBS.
3. Overlay the cells with DRAQ9™ working stock solution (and add any other stains at this point).
4. Incubate for 30 minutes at room temperature. Staining is accelerated at 37°C.
5. Aspirate and apply mountant* and coverslip to slides or tamp excess liquid from microtiter plate wells prior to imaging.

It is also possible to combine DRAQ9™ and formaldehyde into a single "fix & stain" admixed reagent. Prepare a 40 µM working stock solution of DRAQ9™ and an 8% solution of formaldehyde. Mix these together and overlay onto the cells, replacing steps 1-3 of protocol 3.

* BioStatus recommends use of Prolong® Gold (Thermo Fisher Scientific) or Fluoromount-G® (SouthernBiotech)

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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