PRODUCT INFORMATION & DYE TECHNICAL DATA

DRAQ9™

NBP2-81128

For research use only.
Not for diagnostic or therapeutic procedures.

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DRAQ9 ™ TECHNICAL DATA

PRODUCT: DRAQ9 ™
PRODUCT CODES: NBP2-81128

PRESENTATION: blue DMSO solution
STORAGE: store at -20 ºC; use above 20 ºC

DESCRIPTION

DRAQ9 ™ is a novel far-red fluorescing cell permeant probe that labels membranous and vesicular structures in the cytoplasm. It can be used in combination 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.

SPECTRAL CHARACTERISTICS:

Exλ_{max} 605/655 nm Emλ_{max} 698 nm

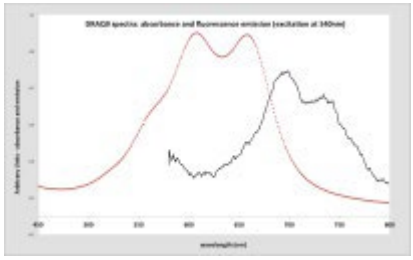

Fig. 1. Spectral profile of DRAQ9 ™

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.

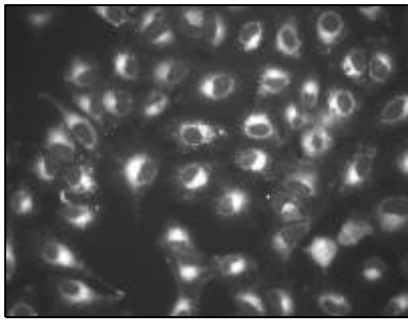

Fig. 2. DRAQ9 ™ labelling of live U2OS cells for 48 h at 2 µM - showing a number of cells in mitosis.

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

Phosphate-buffered saline (PBS, without azide), culture medium, plastic-ware 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 19ºC it is necessary to warm the product vial to above 19ºC 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 -20ºC fridge.

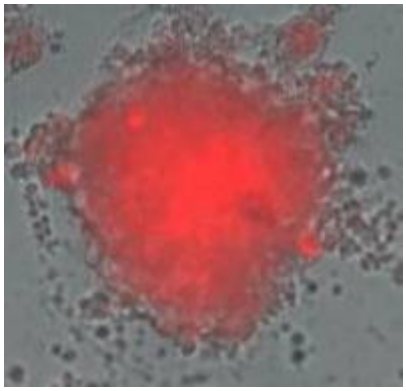

Fig.3. 6-day U2OS spheroid with continuous labeling with DRAQ9 ™ at 2µM

EXAMPLE PROTOCOLS

PROTOCOL 1:

LONG-TERM LIVE CELL TRACKING

A. If cells are already in culture medium simply pipette 11% v/v of the 10X working solution of DRAQ9 ™ into the volume of culture medium in the well or chamber.

Or, preferably:

Add 1 volume of DRAQ9 ™ working stock solution to 9 volumes of culture medium, 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.

B. 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 not intended as a dilution dye and therefore daughter cells will have the same intensity as the parental cells. When replacing culture medium, the medium should contain freshly prepared DRAQ9 ™ at 2µM.

DRAQ9 ™ TECHNICAL DATA

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 / 37ºC.
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 / 37ºC.
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 37ºC.
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" 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.

* Use of Prolong® Gold (Thermo Fisher Scientific) or Fluoromount-G® (SouthernBiotech) is recommended

WHAT YOU SHOULD EXPECT TO SEE

DRAQ9 ™ labels membranous and vesicular structures within the cytoplasm of live or fixed cells, proximal to the nuclear membrane probably a combination of the Golgi apparatus, and endoplasmic reticulum and intracellular vesicular transport system. The pattern is heterogeneous and subtly varies between cell lines. There will be no staining of the nucleus. The pattern of this staining will be altered by any treatment(s) applied to the cells, which can be utilized as a signature of phenotypic response. With image analysis software it may also be possible to mask the nuclear area based on its DRAQ9 ™-negative staining. Note: fixation may alter the labelling pattern from cell type to cell type.

MORE INFORMATION:

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