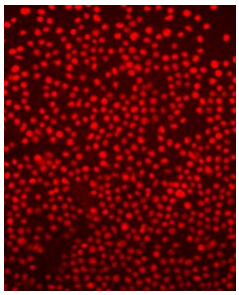
DRAQ5™ in Live End-Point Imaging

Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: LIVE ENDPOINT 002 080323

1. LIVE CELL NUCLEAR COUNTERSTAINING

BACKGROUND



In fluorescence-based microscopy it is normal to preserve cells or tissue sections with formaldehyde or similar fixative. This allows review of the samples at one's convenience and stops the loss or change of the desired biological readout. The fixative works by crosslinking with proteins and causes a mild permeabilisation of the lipid bilayer membranes. However, there are circumstances where fixation is detrimental, for example if the target for analysis leaks into other sub-cellular compartments or leaks out of the cell completely on washing. Alternatively, the fixation process itself may produce artefacts. Similarly, fixation is inappropriate in assays to study drug-receptor interactions as cells are imaged after test antagonists and controls and then again after the reference agonist(s). The same would be true in assays to follow the internalization or processing of a surface-bound molecule (e.g. GPCR). Nonetheless, such live-cell end-point assays, like those on formaldehyde-fixed samples, usually require a nuclear counterstain to locate the individual cells and, in the case of tissue, to give additional information on tissue morphology. Of course, on these samples, it is essential that the nuclear counterstain is fully cell permeant.

WHAT IS THE PROBLEM?

Cell-impermeant DNA dyes are unusable as nuclear counterstains in such assays. The UV-excited dye Hoechst 33342 labels nuclei and is live cell permeant, however on some imaging platforms the coincidental detection of emission from Hoechst and GFP or fluorescein-derived functional probes mean these have to be illuminated sequentially slowing data acquisition and stretching time differences between reading the first and last samples which complicates the performance of live-end end-point assays. Additionally, the binding of Hoechst 33342 to dsDNA is time-sensitive and subject to clearance by MDR-phenotypes and ABCG2 pumps. Hoechst 33342 is often supplied as hard compound and has to be solubilized initially with DMSO and cannot be stored in aqueous form, ready-to-use.

HOW DOES DRAQ5™ HELP?

The far-red, live cell permeant DNA probe DRAQ5™ provides clear nuclear counterstaining in live cells. The bright signal also shows nuclear condensation and fragmentation which indicate deteriorating cell health. DRAQ5™ gives a useful secondary cytoplasmic signal for further cellular segmentation, if required and is achieved by increasing detector gain settings. DRAQ5™ is spectrally separated from the commonly used fluorescent protein tags – e.g. CFP, GFP, YFP, DsRed, mCherry – and the visible-range functional probes which simplifies assay design and allow simultaneous and rapid image acquisition. DRAQ5™'s absorbance profile minimises the risk of FRET-like interactions with other chromophores combined with it. Stoichiometric DNA binding is achieved in minutes, is temporally stable and unaffected by MDR phenotypes.

DRAQ5™ is provided in an aqueous, ready-to-use solution. DRAQ5™ is photo- and chemically-stable, widely compatible with physiological buffers. DRAQ5™ is documented in live end-point imaging.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- rapidly and stably labels all nuclei in live cells
- optimally excited by red laser lines (Ex max 600 & 646 nm)
- compatible with CFP, GFP, YFP and other visible range chromophores
- water-soluble; ready-to-use from the fridge

For a full price list and further information see **www.biostatus.com** or contact us at:
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