DRAQ5™ in Image-Based Screening

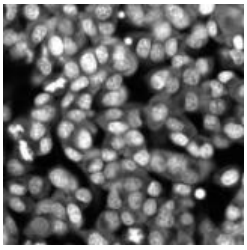
Far-Red Fluorescent Live-Cell Permeant DNA Dye

biostatus

DR5.APPNOTE: IMAGE SCREEN 001 070814

1. NUCLEAR COUNTERSTAINING & NUCL:CYTO SEGMENTATION

BACKGROUND



In most cell-based assays for high content screening (HCS, target-based or phenotypic) fluorescence-based imaging is the preferred approach for adherent cell phenotypes. This allows a number of parameters to be studied simultaneously: a disease-related readout such as a protein translocation, changes in cell numbers and morphology (sentinels for toxicity) or a functional one such as mitochondrial health. In practice, HCS needs a "placeholder" to identify the position of individual cells for measurement using image analysis software. The obvious target for this is staining of the nucleus with a fluorescent DNA binding dye or counterstain.

WHAT IS THE PROBLEM?

As with fluorescence microscopy individual cells should be interrogated with the possibility to segment the cells to their nuclear and cytoplasmic compartments requiring addition of a fluorescent DNA counterstain. The ideal DNA counterstain for nuclei should meet all of the following criteria: show discrete and clear nuclear staining perhaps differentially labelling the cytoplasm; be spectrally separated from commonly used chromophores; work in live or fixed cells; report DNA content; be cross-platform compatible for upstream assay development and allowing transfer to different high content imaging platforms.

There are many DNA binding dyes but few have broad suitability to HCS. DAPI only enters fixed cells and relies upon UV excitation. It undergoes photo-switching to the green emission of FITC / GFP, making review impossible. Propidium iodide only works in permeabilized cells and has insufficient DNA specificity to segment nuclei. Red-emitting dyes TOTO-3 and TO-PRO-3 require permeabilisation and occupy a segment of the visible spectrum useful for rhodamine-based functional dyes. Fixed cell-only dyes limit assay development that would benefit from seeing the biology develop initially in real-time. UV-excited Hoechst 33342 segments nuclei and is live cell permeant, however on many imaging platforms the coincidental detection of emission from Hoechst (and DAPI) and GFP mean that these have to be illuminated sequentially slowing the data acquisition.

HOW DOES DRAQ5™ HELP?

The far-red, live cell permeant DNA probe DRAQ5™ delivers clear nuclear counterstaining in live or fixed cells for ease of transfer from assay development to high-throughput and for live-endpoint assays when fixation is inappropriate. Usefully, it gives a weak secondary signal in the cytoplasm that allows confident segmentation. This feature allows definition of morphological changes indicating compound toxicity. It is spectrally separated from GFP and other visible-range chromophores for easy assay design and simultaneous image acquisition. Due to its stoichiometry with dsDNA the DRAQ5™ nuclear signal can provide cell cycle information. DRAQ5™ is compatible with other image-based screening tools such as *in vitro* toxicology (cell health) assays, as a the DNA counterstain in FISH, tissue-section screening and genotoxicity studies detecting the presence of micronuclei.

Practically, DRAQ5™ is provided in an aqueous, ready-to-use solution and can be admixed with formaldehyde fixative for a single-step fix and stain procedure. It is documented in HCS applications on all imaging platforms.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- rapidly and stoichiometrically labels all nuclei
- optimally excited by red laser lines (Ex max 600 & 646 nm)
- compatible with GFP/FITC, DsRed, rhodamine 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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