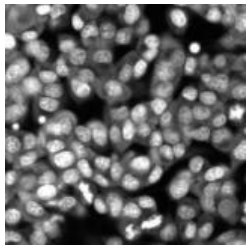
DRAQ5™ in Image-Based *In Vitro* Tox

Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: IMAGE IVT 002 090323

1. NUCLEAR COUNTERSTAINING & NUCL:CYTO SEGMENTATION

BACKGROUND



One of the biggest costs in drug discovery and development has been failure of drug candidates late in the process (or after launch) due to unexpected or idiosyncratic toxicity and risk to patient health. To reduce this, compound libraries are exposed to a battery of mandatory tests, however these are performed when the new chemical entity (NCE) is often well advanced. Recently, in vitro toxicology (IVT) assays have been developed that use physiologically relevant cells (for example, hepatocytes) and measure their health at different NCE concentrations using a few simple parameters from, for example, total cell count, cell/nuclear morphology, plasma membrane failure, mitochondrial membrane potential, reactive oxygen species (ROS) or glutathione (Glu-SH) status. Changes in these parameters are detected using fluorescent functional probes. A live adherent-cell assay is highly amenable to automated fluorescence microscopy as employed in HCS.

WHAT IS THE PROBLEM?

Individual cells need to be interrogated by imaging software, identified by labelling with a fluorescent DNA dye (a.k.a. nuclear counterstain). The ideal nuclear counterstain for image-based IVT should meet all of the following criteria: cell membrane permeant; show discrete and clear nuclear staining and perhaps differentially label the cytoplasm for additional morphological outputs; spectrally separated from commonly used chromophores and not subject to FRET-like bleaching from the NCE; report DNA ultrastructure i.e. condensation or fragmentation; cross-platform compatible for upstream assay development and to allow transfer to different high content imaging platforms.

Cell impermeant DNA dyes are unusable as all-event labelling nuclear counterstains in image-based IVT. UV-excited Hoechst 33342 segments nuclei and is live cell permeant, however on some imaging platforms the coincidental detection of emission from Hoechst and fluorescein-derived functional probes mean these have to be illuminated sequentially slowing data acquisition and stretching time differences between reading the first and last wells on a microtiter-plate. Hoechst 33342 is spectrally incompatible with monochlorbimane (for Glu-SH status), advocated in some hepatotoxicity assays. Hoechst 33342 has been shown to bleach in the presence of a FRET-acceptor compound (e.g. Doxorubicin). 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.

HOW DOES DRAQ5™ HELP?

The far-red, live cell permeant DNA probe DRAQ5™ delivers clear nuclear counterstaining in live cells. The bright signal also shows nuclear condensation and fragmentation. It gives a secondary cytoplasmic signal for further morphometric information, indicative of compound toxicity. DRAQ5™ is spectrally separated from UV-excited, fluorescein- and rhodamine-derived functional probes to ease assay design and allow simultaneous and rapid image acquisition. Its absorbance profile limits the risk of FRET-like interactions with NCE's and functional probes combined with it. DNA binding is temporally stable and unaffected by MDR phenotypes.

Practically, DRAQ5™ is provided in an aqueous, ready-to-use solution. DRAQ5™ is documented in IVT assays.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- rapid, stable labelling nuclei of all cells, independent of state
- optimally excited by red laser lines (Ex max 600 & 646 nm)
- compatible with UV-excited, fluorescein- and rhodamine-derived chromophores
- water-soluble (DMSO-free); highly stable on a HCS automation decks

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