CyTRAK Orange™ in Image-Based *In Vitro* Tox

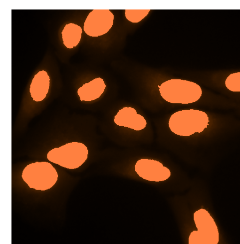
Orange Fluorescent Live-Cell Permeant DNA and Cytoplasmic Dye

biostatus

CO5.APPNOTE: IMAGE IVT 001 150814

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: it must be cell membrane permeant; show discrete and clear nuclear staining and perhaps differentially labelling the cytoplasm for additional morphological outputs; be spectrally separated from commonly used chromophores and not subject to FRET-like bleaching from the NCE; report DNA ultrastructure i.e. condensation or fragmentation; be cross-platform compatible for upstream assay development and allowing 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 microtiterplate. Hoechst 33342 is 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 supplied as hard compound.

HOW DOES CyTRAK Orange™ HELP?

Orange fluorescing live cell permeant DNA probe CyTRAK Orange™ delivers good nuclear counterstaining in live cells. The bright signal shows nuclear condensation and DNA fragmentation. It gives a useful secondary cytoplasmic signal for added morphometric information, indicative of compound toxicity. CyTRAK Orange™ is spectrally separated from UV-excited, fluorescein-derived and red-excited (it is not) functional probes to ease assay design and allow rapid image acquisition. Labelling is temporally stable and unaffected by MDR phenotypes.

Practically, CyTRAK Orange™ is provided in an aqueous, ready-to-use solution.

CyTRAK Orange™ Product Features:

- Orange-fluorescing cell-permeant dsDNA probe (blue / green excited)
- rapidly and stably labels all nuclei
- single-channel dual compartment (nucl:cyto) segmentation
- compatible with UV-excited, fluorescein-derived & red-excited 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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