CyTRAK Orange™ in Image-Based Screening

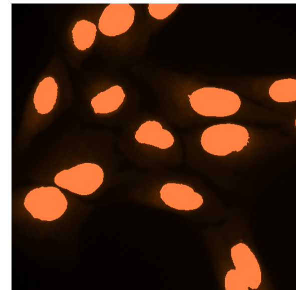
Orange Fluorescent Live-Cell Permeant DNA and Cytoplasmic Dye

biostatus

CO5.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 CyTRAK Orange™ HELP?

The orange-red fluorescing, cell permeant DNA probe CyTRAK Orange™ discretely and differentially stains both nucleus and cytoplasm for confident segmentation. This also allows definition of morphological changes indicating compound toxicity. It works in live or fixed cells for ease of transfer from assay development to high-throughput and for live-endpoint assays when, for example, fixation is inappropriate. It has almost universal instrument compatibility due to its blue/green optimal excitation. It can be combined with UV/violet-excited, GFP and red-excited chromophores (since CyTRAK Orange™ is not). CyTRAK Orange™ also works in image-based screening of tissue-sections. It is detected in an channel centred on 610 nm.

Practically, CyTRAK Orange™ 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.

CyTRAK Orange™ Product Features:

- Orange-fluorescing cell-permeant dsDNA probe
- rapidly and clearly labels all nucleated cells (live or fixed)
- single-channel dual compartment (nucl:cyto) segmentation
- compatible with Horizon BV / BUV, FITC/GFP & red-excited dyes
- 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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