DRAQ5™ in Image-Based Cell Health Assays

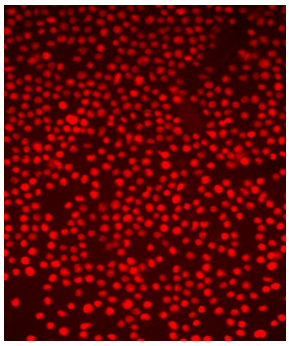
Far-Red Fluorescent Live-Cell Permeant DNA Dye

biostatus

DR5.APPNOTE: IMAGE CELL HEALTH 002 220323

1. TOTAL CELL LABELLING & NUCLEAR COUNTERSTAINING

BACKGROUND



One of the biggest costs in drug discovery and development has been the failure of drug candidates late in the process (or worse after launch) due to unexpected or idiosyncratic toxicity, not to mention the risk to patient health that can result. To reduce this risk compound libraries are exposed to a battery of mandatory tests, however these have been performed when the new chemical entity (NCE) is often well advanced. Recently, new *in vitro* toxicity assays have been developed that use physiologically relevant cells and measure their health under different doses of a NCE using a few simple parameters, for example: total cell count, mitochondrial membrane potential, reactive oxygen species, calcium efflux and glutathione status. A change in each of these parameters is detected using a suitable fluorescent functional probe. A high content screening (HCS) approach using fluorescence-based imaging is preferred for adherent cell phenotypes. In such assays a DNA dye is usually applied to locate and enumerate each cellular event, to determine cell losses and nuclear condensation as measures of cell health.

WHAT IS THE PROBLEM?

These assays are imaged as live cell end-points. Consequently any DNA dye / counterstain used must be cell permeant and show stable binding to nuclei, to be able to report nuclear fragmentation and condensation. Additionally, it must have good spectral compatibility with other fluorescent functional probes required for the assay readout. Additionally, it is helpful if there is a differential cytoplasmic signal. This can be utilised to determine morphological changes that early indicators of toxicity without any other functional changes, which can be useful in assays where cell health is not the primary readout, such in phenotypic drug discovery.

The majority of DNA dyes are cell membrane-impermeant and are therefore incompatible with such assays.

HOW DOES DRAQ5™ HELP?

DRAQ5™ is one of only two proven cell membrane permeant dyes routinely used in HCS, along with the UV-excited dye Hoechst 33342. DRAQ5™ has the advantage of not requiring UV excitation, yet has little spectral overlap with visible range chromophores even allowing combination with rhodamine-derived probes. It is spectrally compatible with simple viability dyes such as propidium iodide, and more sophisticated functional probes such as monochlorbimane (Glutathione status), FITC-Annexin V (membrane inversion), H_2DCFDA (reactive oxygen species), TMRM (mitochondrial membrane potential). DRAQ5™ allows robust cell event counting, while offering nuclear morphology, information on DNA condensation, fragmentation and also, if required, cytoplasmic morphology from a weak differential staining of this compartment (rendered via increased detector gain).

DRAQ5™ is preferentially excited by red wavelengths (Ex max 600 & 646 nm) and detected in any channel above 675 nm, and into the infra-red. Practically, DRAQ5™ is provided in an aqueous, ready-to-use solution usually added as the final step in a staining protocol. It is documented in HCS applications on all imaging platforms.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- rapid, stable, stoichiometric labelling of all nuclei
- optimally excited by red laser lines (Ex max 600 & 646 nm)
- compatible with UV, GFP/FITC, DsRed, rhodamine chromophores
- DMSO-free; refrigerated; stable on automation decks

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