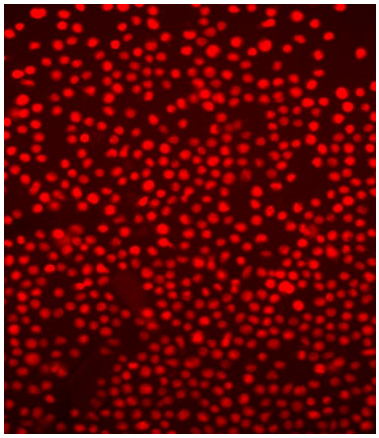
DRAQ5™ in Image-Based Apoptosis Assays

Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: IMAGE APOPTOSIS 002 220323

1. LIVE CELL NUCLEAR COUNTERSTAINING

BACKGROUND



In apoptosis an ordered series of events leads to destruction of a single, cell. The changes that occur can be assayed through cellular morphology, cell numbers, DNA fragmentation, caspase activity (using permeant substrates e.g. FLICA™ probes), mitochondrial membrane potential (e.g. using TMRM), cytochrome C release, plasma membrane inversion (using Annexin V) and permeabilization (using a viability dye) being common choices. These assays are live-cell end-point assays and usually require a nuclear counterstain to demark the individual cells and this needs to be fully cell permeant.

WHAT IS THE PROBLEM?

Cell impermeant DNA dyes are unusable as nuclear counterstains in such assays. The UV-excited dye Hoechst 33342 labels nuclei and is live cell permeant, however on some imaging platforms the coincidental detection of emission from Hoechst and GFP or fluorescein-derived functional probes mean these have to be illuminated sequentially slowing data acquisition and stretching time differences between reading the first and last samples which complicates the performance of live-cell end-point assays. Additionally, the binding of Hoechst 33342 to dsDNA is time-sensitive and subject to clearance by MDR-phenotypes and ABCG2 pumps. Hoechst 33342 is typically supplied as hard compound and has to be solubilized initially with DMSO and cannot be stored in aqueous form, ready-to-use.

HOW DOES DRAQ5 HELP?

The far-red, live cell permeant DNA probe DRAQ5™ provides clear nuclear counterstaining in live cells. The bright signal also shows nuclear condensation and fragmentation that indicate deteriorating cell health. DRAQ5™ gives a useful secondary cytoplasmic signal for further cellular segmentation, if required, by increasing detector gain settings that can also give independent information on cellular morphological responses to apoptotic stimuli. DRAQ5™ is spectrally separated from the commonly used fluorescent protein tags – e.g. CFP, GFP, YFP, DsRed, mCherry – and the visible-range functional probes (e.g. Annexin V-FITC and TMRM) which eases assay design and allows simultaneous, rapid image acquisition. DRAQ5™'s absorbance profile minimises the risk of FRET-like interactions with other chromophores combined with it. Stoichiometric DNA binding is achieved in minutes, is temporally stable and is unaffected by MDR phenotypes.

DRAQ5™ is provided in an aqueous, ready-to-use solution. DRAQ5™ is photo- and chemically-stable, widely compatible with physiological buffers. DRAQ5™ is documented in live end-point imaging.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- rapid, stoichiometric and stable labelling of all nucleated cells
- optimally excited by red laser lines (Ex max 600 & 646 nm)
- compatible with UV-excited and fluorescein- and rhodamine-based chromophores
- water-soluble; refrigerated; stable on automation decks

For a full price list and further information see **www.biostatus.com** or contact us at:
BioStatus Limited
56a Charnwood Road, Shepshed, Leicestershire LE12 9NP United Kingdom
T +44 1509 558 163 | **F** +44 1509 651 061 | **E** enquiry@biostatus.com | **W** www.biostatus.com