

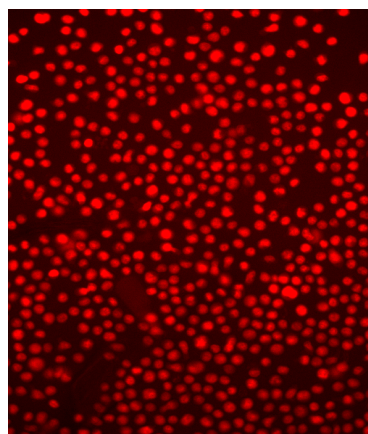


DR5.APPNOTE: IMAGE APOPTOSIS 001 180814

## 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 permeabilisation (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 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 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 allow simultaneous and 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
- ❖ rapidly and stably labels 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; ready-to-use from the fridge



For a full price list and further information see [www.biostatus.com](http://www.biostatus.com) or contact us at:

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