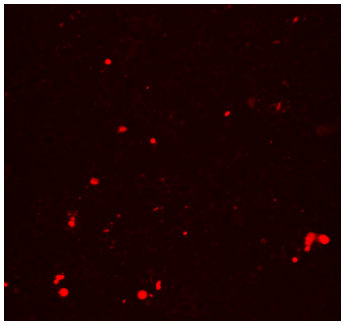
DRAQ7™ in Live End-Point Imaging

Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: LIVE ENDPOINT 001 150814

1. IDENTIFICATION OF DEAD / DAMAGED CELLS

BACKGROUND



In fluorescence-based microscopy it is normal to preserve cells or tissue sections with formaldehyde. However, there are circumstances where fixation is detrimental, for example, if the target leaks into other sub-cellular compartments or leaks out of the cell completely on washing. Alternatively, the fixation process itself may produce artefacts. Similarly, fixation is inappropriate in assays to study drug-receptor interactions as cells are imaged after test antagonists and controls and then again after the reference agonist(s). The same would be true in assays to follow the internalization or processing of a surface-bound molecule (e.g. GPCRs). Nonetheless, in live-cell end-point assays it may be important to identify and quantify dead/damaged cells, which may arise from assay conditions or due to fragility of cells or microtissue being used. In principle, this can be achieved by adding a fluorescent viability dye, typically a cell membrane-impermeant DNA probe that only labels the nuclei of membrane-compromised cells.

WHAT IS THE PROBLEM?

A number of cell membrane-impermeant DNA dyes are candidates to report dead/damaged cells in such live-cell, end-point assays. In fluorescence-based assays it eases assay design if the viability dye is spectrally separated from reagents likely to be used (allowing it to be a default component) importantly GFP, other fluorescent proteins and the cell permeant functional and organelle-specific probes. In this context, propidium iodide (PI) is not ideal due to its broad emission spectrum. Likewise, TOTO-3 and TOPRO-3 have orange/red emission that can occlude the valuable low-red chromophores, depending on filter settings. Meanwhile, DAPI needs equipment that is UV-enabled and due to spectral overlap with GFP and fluorescein-based probes can necessitate duplicate scanning of samples which extends the acquisition time and complicates the timing of measurement.

Long-term or real-time monitoring has become of greater interest with the understanding of idiosyncratic toxicity. None of the viability dyes described above have been validated for long-term monitoring of cell health.

Typically these agents need to be prepared fresh from hard compound and cannot be stored long-term in a ready-to-use aqueous format, and are prone to photobleaching.

HOW DOES DRAQ7™ HELP?

As a far-red DNA-binding viability probe DRAQ7™ immediately avoids spectral overlap with visible-range and UV-excited probes (including Hoechst 33342) allowing it to be used as a default viability probe. It has been validated for long-term, real-time use meaning it can be added to the assay system at any time prior to the measurement. It exhibits extremely low photobleaching. DRAQ7™ only enters cells with compromised membranes and, due to its high dsDNA specificity, reports the nuclear texture of dead and dying cells.

DRAQ7™ is stored in a ready-to-use aqueous format with a very long shelf-life.

DRAQ7™ Product Features:

- far-red fluorescing cell impermeant dsDNA probe
- rapidly and clearly labels only permeabilized cells
- compatible with CFP, GFP, YFP, DsRed proteins and visible range-chromophores
- cross-platform compatible for assay development and transfer
- water-soluble; ready-to-use from the fridge