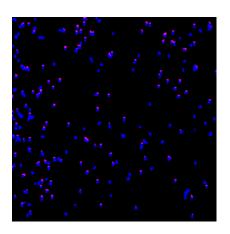


DR7.APPNOTE: IMAGE SCREEN 001 120814

1. DEAD/DAMAGED CELL IDENTIFICATION

BACKGROUND

In most cell-based assays for high content screening (HCS, target-based or phenotypic) fluorescence-based imaging is the preferred. A number of parameters can be studied simultaneously: a disease-related readout such as a protein translocation, often combined with unrelated features that may reflect toxicity of the compounds (or siRNAs) being tested, whether desired (for example, with an anticancer agent) or not. In such live-cell end-point HCS assays it can help to estimate cell death as a measure of toxicity. In more sophisticated temporal- and dose-response assays a real-time measure of cell viability may be advantageous to gain a fuller understanding of cellular sensitivity to a compound. In principle, a simple means of achieving this would be the addition of fluorescent viability dye, typically a cell membrane-impermeant DNA probe that labels the nuclei of membrane-compromised cells.



WHAT IS THE PROBLEM?

A number of cell membrane-impermeant DNA dyes are candidates to report the presence of dead/damaged cells in HCS assays. In fluorescence-based assays it eases assay design if the viability dye is spectrally separated from most reagents likely to be used in HCS (allowing it to be chosen as a default component) and would include GFP, the spectrum of other fluorescent proteins available to tag biologically-relevant 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 rhodamine-based dyes, depending on filter settings. Meanwhile, DAPI, needs equipment that is UV-enabled and due to spectral overlap with GFP and Fluorescein-based probes necessitates duplicate scanning of samples.

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 allowing it to be used as a default viability probe. It has been validated for long-term, real-time use and exhibits extremely low photobleaching. DRAQ7™ only enters cell 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 Horizon BV / BUV, FITC & R-PE dyes
- compensation-free dead cell exclusion (via virtual channel)
- water-soluble; ready-to-use from the fridge

