

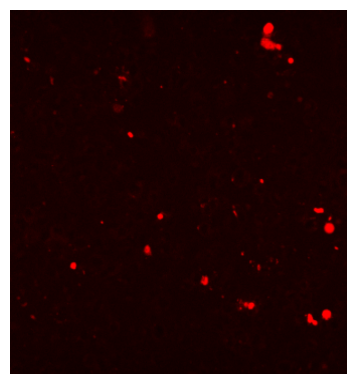


DR7.APPNOTE: IMAGE CELL HEALTH 001 140814

1. IDENTIFYING MEMBRANE-COMPROMISED CELLS IN CELL HEALTH ASSAYS

BACKGROUND

One of the biggest costs in drug discovery and development has been failures of drug candidates late in the process (or after launch) due to unexpected or idiosyncratic toxicity, not to mention the risk to patient health. 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, cell health assays have been developed that use physiologically relevant cells and measure their health under different doses of a compound using a few simple parameters from, for example, total cell count versus plasma membrane failure, mitochondrial membrane potential or calcium efflux. A change in each of these parameters is detected using a suitable fluorescent functional probe. In essence an assay based on these few parameters is highly amenable to fluorescence microscopy.



WHAT IS THE PROBLEM?

A number of cell membrane-impermeant DNA dyes are candidates to report the presence of dead/damaged cells (i.e. with failed membranes) in cell health assays. In fluorescence-based assays it eases assay design if the viability dye is spectrally separated from most reagents likely to be used (allowing it to be chosen as a default component) and would include fluorescein- and rhodamine-derived cell permeant functional and organelle-specific probes e.g. H₂DCFDA and TMRM, respectively. 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. Meanwhile, DAPI needs equipment that is UV-enabled and due to spectral overlap with fluorescein-based probes can necessitate duplicate scanning of samples, which is inadvisable in a live-cell experiment. Additionally, UV-excited Hoechst 33342 is commonly used for the total cell count.

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 the agents above 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 cell health assays 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 membrane-compromised cells
- ❖ compatible with UV-excited, fluorescein- and rhodamine-based probes
- ❖ validated for long-term, real-time cell health monitoring
- ❖ water-soluble; ready-to-use from the fridge



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

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