

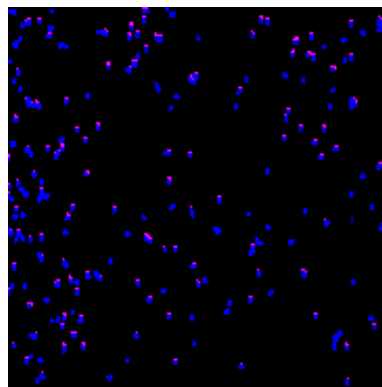
# DRAQ7™ in Image-Based *In Vitro* Tox

## Far-Red Fluorescent Live-Cell Impermeant DNA Dye

### 1. IDENTIFYING MEMBRANE-COMPROMISED CELLS

#### BACKGROUND

A persistent cost in drug development is the late-stage failure of drug candidates due to unexpected or “idiosyncratic” toxicity, with the obvious risk to patients. To reduce this risk compound libraries are exposed to a battery of mandatory tests, however these are 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 compound doses using a few simple parameters from, for example, total cell count vs. plasma membrane failure, mitochondrial membrane potential, reactive oxygen species (ROS) or glutathione (Glu-SH) health. Changes in any of these can be detected via fluorescent functional probes. An assay based on such parameters is highly amenable to automated fluorescence microscopy as employed in HCS.



#### 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 *in vitro* toxicity assays. In fluorescence-based assays it eases assay design if such a viability dye is spectrally separated from most reagents likely to be used (allowing it to be chosen as a default component), that might include fluorescein- and rhodamine-derived cell permeant functional and organelle-specific probes e.g. H<sub>2</sub>DCFDA 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 viability assays and 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
- ❖ rapid, clear labelling of only membrane-compromised cells
- ❖ compatible with UV-excited, fluorescein- and rhodamine-based probes
- ❖ validated for long-term, real-time *in vitro* toxicity monitoring
- ❖ water-soluble; DMSO-free; easy-to-use from the fridge



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