

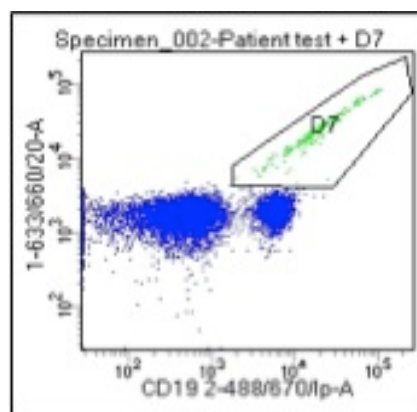


DR7.APPNOTE: FLOW IVT 001 060814

1. IDENTIFYING MEMBRANE-COMPROMISED CELLS IN *IN VITRO* TOXICITY

BACKGROUND

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



WHAT IS THE PROBLEM?

For the measurement of plasma membrane integrity traditional viability dyes (DAPI, PI, 7-AAD) have proved to be incompatible despite the assay's simplicity. They are spectrally incompatible, have high non-specific binding that drifts upwards with time or exhibit low s:n. These characteristics make them generally unsuitable. More recent alternatives include TOTO-3 and TO-PRO-3 but these are costly to run in the volumes associated with non-adherent cells such as leukocytes. The chemistry of these dyes means they are supplied in hard compound form and require DMSO to get them into solution and cannot be stored in aqueous solution for extended periods.

HOW DOES DRAQ7™ HELP?

As a far-red DNA-binding viability dye DRAQ7™ immediately alleviates the problems of spectral overlap with functional probes such as TMRM, Fluor-4 and Hoechst 33342.

DRAQ7™ rapidly stains cells with compromised membranes giving an excellent separation between intact and damaged/dead cells. It is cost-effective and reliable in such assays.

Uniquely, DRAQ7™ is compatible with long-term, real-time cell health assays meaning that cells can be sampled serially to determine temporally-related deterioration in cell health. This also means that it can be added to cells for end-point analysis without affecting the protocol.

DRAQ7™ has excellent chemical- and photo-stability. Accordingly, DRAQ7™ is supplied in a ready-to-use aqueous format with a very long shelf-life making it amenable to high-throughput applications where reagents are pipetted from bulk quantities on robotic platforms, often with limited environmental control.

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



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

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