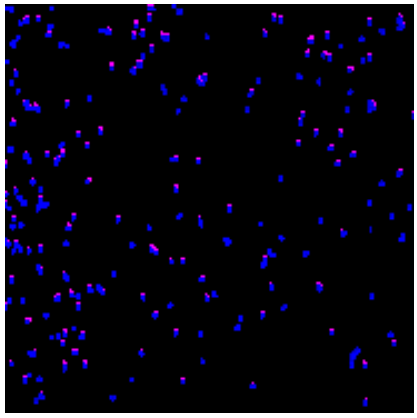
DRAQ7™ in Plate-based Cytometry

Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: PLATE CYTOMETRY 002 230323

1. DEAD CELL EXCLUSION

BACKGROUND



Microplate- and slide-based cytometers (e.g. TTP Labtech's Acumen eX^3, Nexcelom's Celigo S) utilising laser scanning combine morphological (textural) capabilities of fluorescence-based imaging with cell-by-cell multi-parameter analysis of cytometers. These are particularly valuable for adherent cell types that are not readily compatible with flow cytometry.

Exclusion of dead/damaged cells is often needed for accurate cell phenotyping by plate-/slide-based cytometry. Enumeration of dead cells can determine when samples are damaged beyond a level to allow robust analysis (often due to poor storage/transport or excessive processing). Dead cells can bind antibody in an unspecific manner presenting erroneous phenotypes. Typically, dead cells are labelled with a fluorescent dye that only enters membrane-compromised cells (including apoptotic/damaged/dead cells) and usually binds to DNA (so-called viability dyes).

WHAT IS THE PROBLEM?

To achieve this, traditional viability dyes such as propidium iodide (PI) or DAPI have been used. However, each has challenges associated with it.

PI progressively equilibrates with intact cells in long-term experiments confounding the ability to set a threshold for positive cells. Even more importantly, PI's fluorescence completely overlaps with R-PE, a widely used bright chromophore. DAPI is UV-/violet-excited and its fluorescence occludes the new BUV and BV chromophores that would extend the capacity of the current instrumentation and allow re-design of current antibody/chromophore panels, but is known to be semi-permeant.

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

HOW DOES DRAQ7 HELP?

As a far-red DNA-binding viability dye DRAQ7 immediately alleviates the problems of spectral overlap with R-PE (PI) and the BUV, BV and violet dyes (DAPI).

DRAQ7™ gives a distinct nuclear stain (pink, in the figure) in dead/damaged/apoptotic cells and bright punctate nuclear staining in cells experiencing DNA fragmentation.

DRAQ7™ is truly cross-platform compatible allowing assays to be transferred to conventional flow cytometers, fluorescence microscopes and HCS imaging platforms. DRAQ7™ is stored in a ready-to-use aqueous format with a very long shelf-life.

For a full price list and further information see **www.biostatus.com** or contact us at:
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2. IDENTIFYING MEMBRANE-PERMEABILIZED CELLS IN APOPTOSIS ASSAYS

BACKGROUND

In apoptosis an ordered series of events leads to destruction of a single cell. The changes that occur can be assayed through DNA fragmentation, caspase activity (using permeant substrates e.g. FLICA™ probes), mitochondrial membrane potential (e.g. using TMRM), cytochrome C release, plasma membrane inversion (using Annexin V) and permeabilization being common choices. Plasma membrane permeabilization is a late hallmark of apoptosis identified with a viability probe. This is combined with other apoptotic features to give a temporal context and to identify cells permeabilized non-apoptotically (e.g. mechanically).

WHAT IS THE PROBLEM?

To achieve this, traditional viability dyes such as propidium iodide (PI) or DAPI have been used. However, each has challenges associated with it.

PI progressively equilibrates with intact cells resulting in these cells drifting right on plots confounding the ability to reliably set a gate for positive cells. Even more importantly, PI's fluorescence completely overlaps with TMRM and JC-1, commonly used mitochondrial membrane potential probes. DAPI is UV/violet excited and cannot be combined with UV-excited Hoechst 33342 probe commonly used as an "all-event" marker in apoptosis assays. Photo-switching of DAPI and photo-bleaching of PI as well as evidence of semi-permeance make these DNA binding agents incompatible with long-term assays. Typically these agents need to be prepared fresh from hard compound and not stored long-term, i.e. ready-to-use.

HOW DOES DRAQ7™ HELP?

As a far-red DNA-binding viability dye DRAQ7™ immediately alleviates the problems of spectral overlap with TMRM (PI) and UV-excited dyes like monochlorbimane (Glu-SH probe) and Hoechst 33342. Thus, DRAQ7™ can be combined with Hoechst 33342, Annexin V-FITC, TMRM, for example. DRAQ7™ is highly chemically and photo- stable. Uniquely, it has also been shown to be compatible with time-lapse cell health assays meaning that cells can be sampled serially to determine the temporal development of the apoptotic events in the assay system, over many days. DRAQ7™ is stored in a ready-to-use aqueous format with a very long shelf-life making it convenient and easy to use.

DRAQ7™ Product Features:

- far-red fluorescing cell impermeant dsDNA probe
- rapid, clear labelling of only the leaky/dead cells
- compatible with BV- / BUV- like, FITC & R-PE dyes
- uniquely permits viability monitoring in long-term assays
- water-soluble; refrigerated; ready-to-use