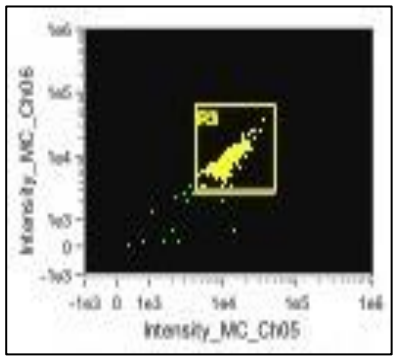
DRAQ7™ in Imaging Flow Cytometry

Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: IMAGING FCM 003 230323

1. DEAD CELL EXCLUSION

BACKGROUND



Exclusion of dead/damaged cells is often needed for accurate cell phenotyping by imaging flow 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 also bind antibody in an unspecific manner and therefore present erroneous phenotypes that interfere with analysis of intact cells. 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), 7-AAD or DAPI have been used. However, each has challenges associated with it.

PI progressively equilibrates with intact cells resulting in these cells drifting right confounding the ability to reliably set a gate for positive cells. Additionally, PI's broad fluorescence completely overlaps with R-PE, a widely used bright chromophore.

DAPI is UV/violet excited, which is described as semi-permeant and its fluorescence occludes the BUV- / BV- like chromophores that may extend the capacity of the Imagestream™ and FlowSight™ instruments and allow re-design of current antibody-chromophore panels.

7-AAD suffers for poor segregation of intact cells from intermediate and fully leaky (dead) cells.

Typically, 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 (by PI), BUV- / BV- like chromophores and violet dyes (DAPI).

DRAQ7™ gives a distinct nuclear stain in dead/damaged/apoptotic cells and bright punctate nuclear staining in cells experiencing DNA fragmentation.

DRAQ7™ is truly cross-platform compatible: assays can be easily transferred to conventional flow cytometers, fluorescence microscopes and high content 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 by non-apoptotic means.

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 making it difficult to reliably set an intensity for positive cells. PI's RNA-associated fluorescence (in the cytoplasm) completely overlaps with TMRM, a commonly used mitochondrial membrane potential probe. 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. Evidence of semi-permeance make these DNA binding agents incompatible with monitoring long-term assays. Typically these agents need to be prepared fresh from hard compound and cannot be stored long-term 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 (DAPI). Unlike PI, the staining pattern of DRAQ7™ is restricted to the nucleus, important for imaging flow cytometry applications. DRAQ7™ can be combined with Hoechst 33342, Annexin V-FITC, TMRM, for example. DRAQ7™ is extremely chemically- and photo-stable. Uniquely, it has also been demonstrated in long-term, real-time cell health assays meaning that cells can be sampled serially to determine the temporal development of the apoptotic events in the assay system. 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, nuclear labelling, only in permeabilized cells
- compatible with all UV/violet -excited and visible-range chromophores
- compensation-free dead cell exclusion (via virtual channel)
- water-soluble; refrigerated; easy-to-use