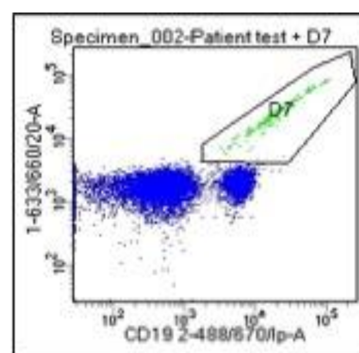
DRAQ7™ in Flow Cytometry

Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: FLOW CYTOMETRY 002 220323

1. DEAD CELL EXCLUSION

BACKGROUND



Exclusion of dead/damaged cells is usually needed for accurate cell phenotyping by 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 bind antibody in an unspecific manner and therefore can present incorrect phenotypes that interfere with analysis of intact cells. An example of a sample requiring estimation / exclusion of dead cells is the ISHAGE protocol for CD34+ stem cell enumeration from mobilised blood, bone marrow or cord blood. Typically, to achieve this, dead cells are labelled with a fluorescent dye that can only enter 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. 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 Horizon Brilliant ™ UV and violet chromophores that would extend the capacity of the current instrumentation and allow re-design of current antibody/chromophore panels.

7-AAD has been shown to exhibit very poor segregation of the three populations: intact, live cells; newly leaky cells; and dead cells, making gate placement troublesome.

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 (PI) and the new Horizon Brilliant™ UV and violet dyes (DAPI). Further, DRAQ7™ has a unique ability on dual laser (blue/red) equipped cytometers for dead cells to be displayed in a "virtual" channel or diagonal gate that avoids any compensation issues. This means it can be added to pre-existing antibody panels without re-design or occupancy of useful channels.

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), 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 on plots confounding the ability to reliably set a gate for positive cells. Even more importantly, PI's fluorescence 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. 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. 7-AAD shows poor separation between intact and leaky/dead cells making multi-parameter assays more difficult. Typically, these need to be prepared fresh from hard compound and cannot be stored 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 extremely chemically and photo-stable. Uniquely, it has also been shown to be compatible with 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
- 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

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