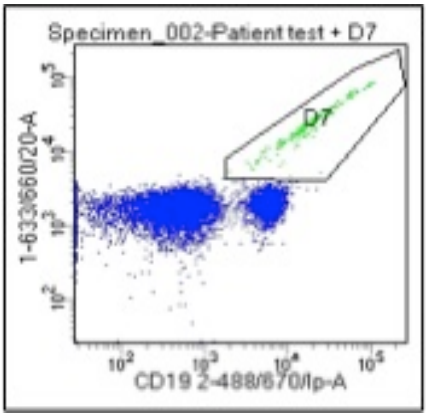
DRAQ7™ in HTFC-Based Screening

Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: FLOW SCREEN 001 050814

1. DEAD CELL EXCLUSION

BACKGROUND



Exclusion of dead/damaged cells is often 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 also have the unhelpful ability to bind antibody in an unspecific manner and therefore can present erroneous phenotypes that interfere with analysis of the 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) 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, which is unacceptable in a high throughput setting. Even more importantly, PI's fluorescence completely overlaps with R-PE, a widely used bright chromophore. DAPI is UV/violet excited (if such is available) 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.

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). Uniquely, DRAQ7™ has the ability on dual laser (blue/red) equipped cytometers for dead cells to be displayed in a "virtual" channel that avoids any compensation issues. This means that it can be added to 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, ideal for high throughput applications requiring automation and bulk preparation.

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, JC-1), cytochrome C release, plasma membrane inversion (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 suitable 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 events drifting right on plots making it difficult to set a gate for positive cells that is temporally stable. PI's fluorescence completely overlaps with TMRM and JC-1, commonly used mitochondrial membrane potential probes. DAPI is UV-/violet- excited (if even available) and cannot be combined with the UV-excited Hoechst 33342 probe commonly used as an "all-event" marker. Photo-switching of DAPI and photo-bleaching of PI as well as evidence of semi-permeance make these DNA binders incompatible with long-term assays. These agents need to be prepared fresh from hard compound and can't be stored long-term, ready-to-use for high throughput.

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 an assay system, while revealing an ideal property for high throughput applications when working with live cells. DRAQ7™ is stored in a ready-to-use aqueous format with a very long shelf-life making it convenient, easy to use and compatible with large-scale screens.

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