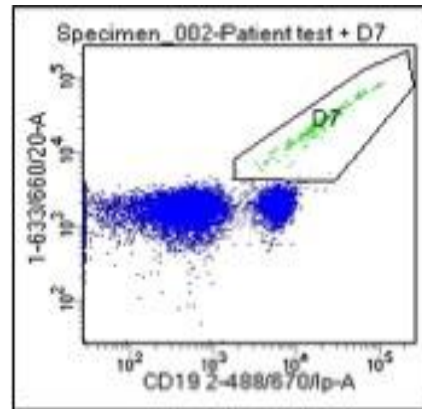
DRAQ7™ in HTFC-Based Screening

Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: FLOW SCREEN 002 240323

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 can also bind antibody in an unspecific manner and so present erroneous 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 so that these cells drift right confounding the ability to reliably gate for positive cells, which is unacceptable in high throughput, automation. 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 BV-/BUV-like chromophores that can extend the capacity of 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, again important in automated approaches.

Typically, these agents must be prepared fresh from hard compound and cannot be stored long-term in ready-to-use aqueous format, for the processing of large sample numbers.

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.

For a full price list and further information see **www.biostatus.com** or contact us at:
BioStatus Limited
56a Charnwood Road, Shepshed, Leicestershire LE12 9NP United Kingdom
T +44 1509 558 163 | **F** +44 1509 651 061 | **E** enquiry@biostatus.com | **W** www.biostatus.com

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), 7-AAD or DAPI have been used. However, each has challenges associated with it.

PI progressively equilibrates with intact cells so that event drift 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 often used to mark "all-events". Photo-switching of DAPI and photo-bleaching of PI as well as evidence of semi-permeance make them incompatible with long-term assays. 7-AAD has been shown to give poor segregation of the three populations: intact, live cells; newly leaky cells; dead cells, making gate placement troublesome, again important in automated approaches. These agents need to be prepared fresh from hard compound and can't be stored long-term, for high throughput use.

HOW DOES DRAQ7™ HELP?

As a far-red DNA-binding viability dye DRAQ7™ avoids spectral overlap with TMRM and UV-excited dyes like monochlorbimane (Glu-SH probe) and Hoechst 33342. Then, DRAQ7™ can be combined with Hoechst 33342, Annexin V-FITC, TMRM, etc. DRAQ7™ is highly chemically and photo-stable. Uniquely, it has also been shown to be compatible with long-term, real-time cell health assays meaning cells can be sampled serially to determine temporal development of apoptotic events, while revealing an ideal property for high throughput applications and live cells. DRAQ7™ is stored in ready-to-use aqueous format with a 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
- rapid, clear labelling of only the permeabilized cells
- compatible with BUV-/BV-like, vis.-range dyes and FPs
- compensation-free dead cell exclusion (via diagonal gating)
- DMSO-free; ready-to-use; stable on automation decks

For a full price list and further information see **www.biostatus.com** or contact us at:
BioStatus Limited
56a Charnwood Road, Shepshed, Leicestershire LE12 9NP United Kingdom
T +44 1509 558 163 | **F** +44 1509 651 061 | **E** enquiry@biostatus.com | **W** www.biostatus.com