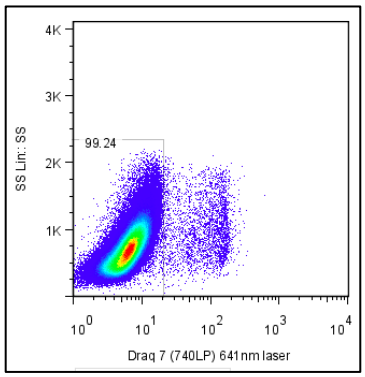
DRAQ7™ in Cell Sorting (FACS)

Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: CELL SORTING 002 100523

1. DEAD CELL EXCLUSION / "DUMP" CHANNEL GATING

BACKGROUND



Exclusion of dead/damaged cells is normally needed in cell sorting. Initial enumeration of dead cells can determine when samples are damaged beyond a level to continue sorting. Dead cells also have high capacity to bind antibody unspecifically and therefore can present erroneous phenotypes that can compromise sorting purity of intact cells. Ideally, then, dead cells are negatively sorted into a so-called "dump" channel, usually along with any other phenotypic markers of unwanted cells.

Typically, to achieve this, dead cells would be 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). However, in many cases dead cells are identified on the basis of their approximate position on a scatter plot due to concerns with the potential toxicity of the viability dye, or at the very least the concern that the dye achieves equilibration at low-level in all the cells irrespective of their membrane integrity.

WHAT IS THE PROBLEM?

Traditionally, 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. 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 (and their analogues) that would extend the capacity of the current instrumentation and allow re-design of current antibody/chromophore panels. DAPI is described as semi-permeant to cells.

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

HOW DOES DRAQ7™ HELP?

As a far-red DNA-binding viability dye DRAQ7™ overcomes the problem of spectral overlap with R-PE (PI) and new BUV and BV dyes (DAPI). DRAQ7 can be combined with -Cy5.5 or -Cy7 fluorophores in a common dump channel to simplify the gating strategy. Also, DRAQ7™ has the unique ability on dual laser (blue/red) equipped cytometers for dead cells to be dump sorted via a "virtual" channel avoiding compensation. This means it can be added to existing antibody panels without re-design or to free useful channels.

DRAQ7™ has been demonstrated for long-term assays allowing it to be used in cell sorting applications where cell viability and avoidance of toxicity with the viability dye are equally important.

DRAQ7™ is stored in a ready-to-use aqueous format with a very long shelf-life.

DRAQ7™ Product Features:

- far-red fluorescing cell impermeant dsDNA probe
- rapidly and clearly labels only permeabilized cells
- compatible with BV / BUV, FITC & R-PE dyes
- compensation-free dead cell exclusion (via virtual channel)
- ultra-low toxicity; water-soluble; ready-to-use from the fridge

For a full price list and further information see **www.biostatus.com** or contact us at:
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