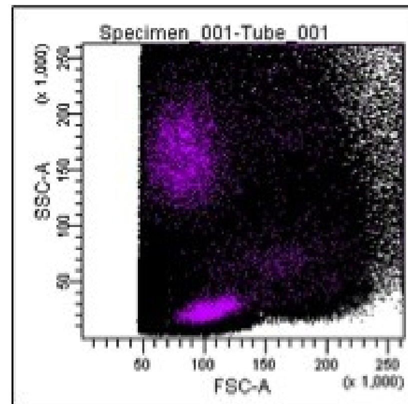
BIOSTATUS

DRAQ5™ in HTFC-Based Screening

Far-Red Fluorescent Live-Cell Permeant DNA Dye

1. NUCLEATED CELL GATING

BACKGROUND



Normal blood and bone marrow samples contain a mixture of nucleated cells and enucleated cells (mature erythrocytes i.e. RBCs, and platelets). RBCs can interfere with analysis of nucleated cells, especially in flow cytometry where they complicate and slow phenotypic analysis. The most common solution is to osmotically shock the enucleated cells with NH_4Cl – RBC lysis. After this, the nucleated cells are pelleted by centrifugation, washed, counted and resuspended for use.

Similarly, with cultured cells it is useful to be able to gate out debris that can range from subcellular fragments to whole cells with fragmenting nuclei.

WHAT IS THE PROBLEM?

There are many potential risks from RBC lysis: additional time required; release of debris into the sample that can aggregate with leukocytes; inconsistent results; possible lysis of erythroid precursors; non-specific cell losses during washing procedures. The relative importance of these may vary but cell loss would be of concern with mouse tail vein samples or in the detection of extremely rare cells such as circulating tumor cells, for example.

It is difficult to set a gate to exclude any non-viable cells with fragmenting nuclei from intact viable cells. These non-viable cells may interfere with the analysis, particularly of rare cells.

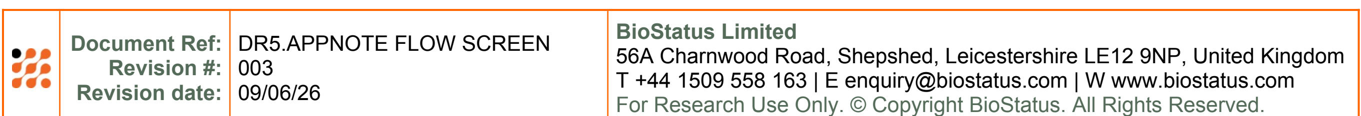
HOW DOES DRAQ5™ HELP?

The presence of nuclear DNA allows differentiation between nucleated and enucleated cells. DRAQ5™ is a live-cell permeant dsDNA-specific probe that efficiently and stably labels nucleated cells. DRAQ5™ fluoresces in the far-red when excited by blue or red laser on standard flow cytometer. The signal is detected in any channel above 675 nm, and preferably centred around peak emission at 697 nm. This signal is then used to select exclusively or "gate" the nucleated cells without the complexity and risk associated with RBC lysis. Nucleated cells may include rare tumor or endothelial cells not present in healthy blood or marrow. Such labelling of the nucleated cells also broadens the choice of RBC lytic techniques if these are, nonetheless, deemed necessary.

The DRAQ5™ signal can identify non-viable/apoptotic cells with sub-G_1/G_0 DNA content (with fragmenting nuclei) and these can be excluded with more confidence from intact cells.

Additionally, the DRAQ5™ signal can identify cell doublets (plot peak area vs. peak width) and DNA content of each cell (see below). The far-red fluorescence of DRAQ5™ means that it can be combined with most visible range chromophores with limited or no spectral overlap.

2. DNA CONTENT (CELL CYCLE) ANALYSIS



BACKGROUND

The cell cycle is a series of events that occur when a cell divides and replicates into two daughter cells. Cell cycle regulation depends upon processes pivotal to cell survival. During cell cycle the quantity of DNA increases from 2N (G1 phase) to 4N (G2) via the S (synthesis) phase. Perturbation of cell cycle can lead to cell cycle arrest or uncontrolled cell division, as in cancer, while cells with DNA below 2N are typically apoptotic, or either side of 2N, aneuploid. DNA content in each nucleated cell in a population can be measured by labelling the nuclear DNA with a fluorescent DNA dye, most commonly propidium iodide (PI). Signals are plotted to create a DNA profile for the population giving information on proliferative status and cell cycle arrest, with phenotyping where required, to assess the impact of different treatments to cells.

WHAT IS THE PROBLEM?

Using PI to measure DNA content requires that nucleated cells are separated from a blood or bone marrow sample by ficoll density centrifugation. Thereafter, the isolated cells (or cultured cells) are then permeabilized (to allow dye entry) and treated with RNase (PI non-specifically binds to RNA). Spectrally PI is excited by the blue laser and detected around 610 nm, overlapping with R-PE a very bright and commonly used antibody conjugated dye. Alternatively, one can use the live-cell permeant DNA probe Hoechst 33342, excited by UV light. However, UV sources are less common on modern flow cytometers, replaced by violet sources driven by the emergence of new classes of violet–excited antibody labels.

HOW DOES DRAQ5™ HELP?

As a live-cell permeant DNA probe DRAQ5™ can be applied directly to complex samples such as blood or bone marrow (as described above), as the last step prior to analysis and without washing. No complex processing, such as RBC lysis, ficoll separation and permeabilization, is needed. Its high dsDNA specificity avoids the need for RNase treatment. It is possible to get DNA profiles with the minimum disturbance to the sample, especially important for precious, small samples or where there is a risk of uncontrolled cell losses. The far-red fluorescence is temporally stable. It is spectrally compatible with most visible-range chromophores for multi-colour analysis. It is excited by blue or red laser lines, found on most flow cytometers.

For convenience DRAQ5™ is supplied in an aqueous ready-to-use formulation.

DRAQ5™ Product Features:

- far-red fluorescing live-cell permeant dsDNA probe
- water-soluble (DMSO-free); highly stable on automation decks
- rapid, stoichiometric labelling of all nucleated cells
- readily compatible with BV / BUV, FITC & R-PE dyes
- excited by red or blue laser lines

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