DRAQ5™ in FC-Based *In Vitro* Tox

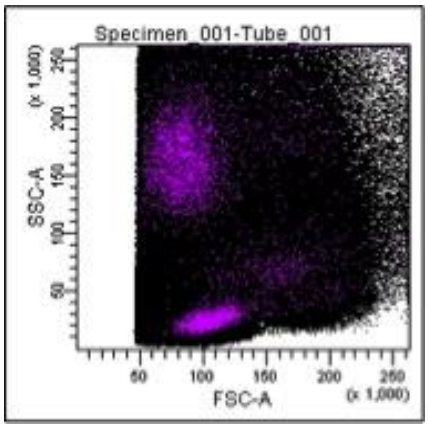
Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: FLOW IVT 002 090323

1. NUCLEATED CELL GATING

BACKGROUND

In vitro toxicology testing on blood and bone marrow is an emerging field. These samples contain a mixture of nucleated cells and enucleated cells and there are around 40 platelets and 400 erythrocytes for every nucleated cell. These can interfere with analyses of the 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 – known as RBC lysis. After this, the nucleated cells are pelleted by centrifugation, washed, counted and resuspended for use.



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 (causing a skewing of the myeloid:erythroid ratio); non-specific cell losses during washing procedures. The relative importance of these may vary but obviously these can impact the performance of robust assays and use of automation.

HOW DOES DRAQ5™ HELP?

The presence of genomic DNA is a simple way to differentiate between nucleated and enucleated cells. DRAQ5™ is a live-cell permeant dsDNA-specific probe that efficiently and stably labels nucleated cells. It is added to diluted whole blood or bone marrow, mixed and briefly incubated. DRAQ5™ fluoresces in the far-red when excited by blue or red laser on any standard flow cytometer. The signal is detected in any channel above 675 nm, preferably centred on 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.

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

For convenience DRAQ5™ is supplied in an aqueous ready-to-use formulation and is amenable to routine applications and automated procedures.

2. DNA CONTENT (OR 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 that are pivotal to cell survival. During the cell cycle the quantity of DNA increases from 2N (G1 phase) to 4N (G2 phase) via the S (synthesis) phase. Perturbation of the cell cycle by toxic insult can lead, for example, to cell cycle arrest or uncontrolled cell division, or DNA fragmentation (e.g. in apoptosis) with cells having DNA below 2N. The quantity of DNA in each nucleated cell in a population can be measured by labelling the nuclear DNA with a fluorescing DNA dye, most commonly propidium iodide (PI). The signals are plotted to create a DNA profile for the population giving information on the proliferative status, usually alongside phenotyping.

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 that is 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 or ficoll separation and permeabilization are needed. The high dsDNA specificity of DRAQ5™ avoiding 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 loss. Far-red fluorescence makes DRAQ5™ 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
- rapid, stoichiometric labelling all nucleated cells
- compatible with BV / BUV, FITC & R-PE dyes
- water-soluble (DMSO-free); easy-to-use from the fridge
- excited by red or blue laser lines