DRAQ5™ in Cell Cycle Analysis

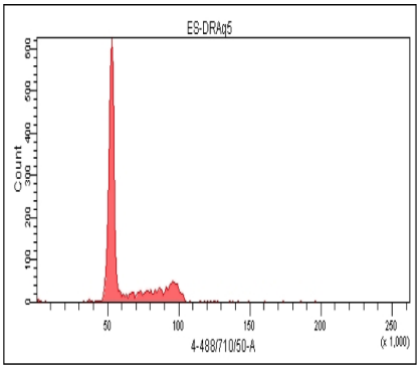
Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: CELL CYCLE 003 060323

1. 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 can lead, for example, to cell cycle arrest or uncontrolled cell division, as in cancer, while cells with DNA below 2N are typically in apoptosis. 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 or by RBC lysis. The latter can lead to the uncontrolled lysis and therefore loss of cells such as late erythroblasts. 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, due to the emergence of new classes of violet–excited antibody labels and the use of harmful UV is not desirable if live-cell preparations are being analysed.

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, rapidly labelling the nucleated cells, as the last step before analysis and without washing. No complex processing such as RBC lysis or ficoll separation and permeabilization are needed. Its high dsDNA specificity avoids the need for RNase treatment. Nucleated cells are gated on the basis of their DRAQ5 signal and the doublets excluded by plotting area versus width for the DRAQ5 cell signals. It is then 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. 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 modern 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; easy-to-use from the fridge
- rapidly and stoichiometrically labels all nucleated cells
- readily compatible with BV / BUV, FITC & R-PE dyes
- excited by red-to-blue laser lines

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