DRAQ5™ in FC-Based Apoptosis Assays

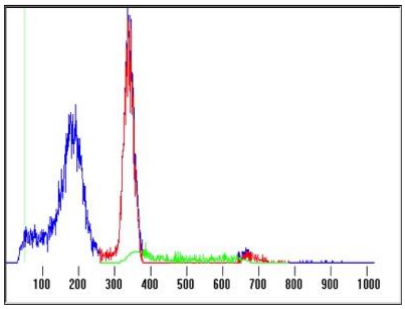
Far-Red Fluorescent Live-Cell Permeant DNA Dye

biostatus

DR5.APPNOTE: FLOW APOPTOSIS 002 220323

1. SUB-G_1/G_0 DNA CONTENT 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 to cell cycle arrest or uncontrolled cell division, as in cancer, while cells with DNA below 2N are typically in apoptosis, this is the, so-called, sub-G_1/G_0 peak (as shown in the figure, labelled in blue to the left of the G_1/G_0 peak). 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 (anchored on the G_1 and G_2 peaks) for the population giving information on cells experiencing DNA fragmentation, usually alongside phenotyping.

WHAT IS THE PROBLEM?

Using PI, 7-AAD or DAPI to measure DNA content requires that nucleated cells are permeabilized (to allow dye entry) and, in the case of PI, RNase treatment to overcome non-specific binding to RNA. The risk from permeabilization is that (apoptotic) DNA fragments are washed away and lost to the analysis. Also, 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 with violet sources due to the emergence of new classes of violet–excited antibody labels.

HOW DOES DRAQ5™ HELP?

As a live-cell permeant DNA dye DRAQ5™ can be applied directly to complex samples such as blood or bone marrow or cultured cells, as the last step prior to analysis and without further washes. No complex processing such as RBC lysis or ficoll separation and permeabilization are needed since with blood or bone marrow one can gate on the DRAQ5 positive events to exclude enucleated cells. High dsDNA specificity avoids the need for RNase treatment. It is possible to get sub-G_1/G_0 DNA profiles with minimum disturbance to the sample, especially important for precious, small samples or where there is a risk of uncontrolled cell losses or of fragmented DNA.

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 of all nucleated cells
- excited by red or blue laser lines
- compatible with BV / BUV, FITC & R-PE dyes
- water-soluble; easy-to-use, direct from the fridge

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