

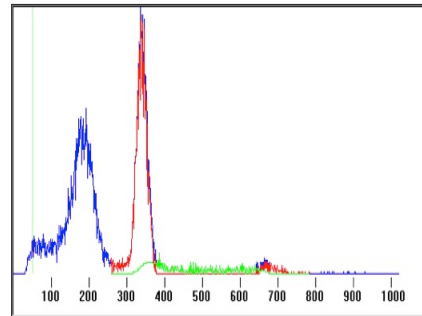


1. SUB-G₁/G₀ DNA CONTENT ANALYSIS

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, for example, 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-G₁/G₀ peak. The quantity of DNA in each nucleated cell in a population can be measured by labeling 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 G₁ and G₂ peaks) for the population giving information on cells experiencing DNA fragmentation, usually alongside phenotyping.

WHAT IS THE PROBLEM?

Using PI or DAPI to measure DNA content requires that nucleated cells 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 the 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 that is 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 probe 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 any washing steps. 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 the nucleated cells. Its high dsDNA specificity avoids the need for RNase treatment. It is possible to get sub-G₁/G₀ 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
- ❖ rapidly and stoichiometrically labels all nucleated cells
- ❖ excited by red or blue laser lines
- ❖ compatible with Horizon BV / BUV, FITC & R-PE dyes
- ❖ water-soluble; ready-to-use from the fridge

