BIOSTATUS

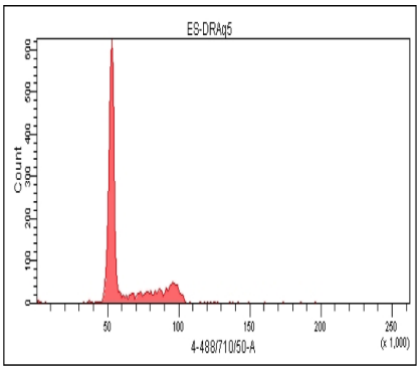
DRAQ5™ in Cell Sorting (FACS)

Far-Red Fluorescent Live-Cell Permeant DNA Dye

1. SINGLE CELL SORTING FOR GENOMICS AND TRANSCRIPTOMICS

BACKGROUND

The cell cycle describes cell division and replication into two daughter cells, when the quantity of gDNA increases from 2N (G_1 phase) to 4N (G_2 phase) via the S (synthesis) phase. gDNA in each nucleated cell can be measured with a fluorescent DNA-binding dye, using flow cytometry. The signals are plotted to create a DNA profile for the population giving a snapshot of the proliferative status, usually alongside phenotyping, that together can be used for cell sorting and downstream genomic, transcriptomic or even proteomic analysis and thereby allowing study of molecular features of individual 2N cells ("singlet events") and when required, in the context of the cell cycle position (e.g. G_S or $G_{2/M}$) of the portion of cells within the sorting parameters selected.



WHAT IS THE PROBLEM?

Most DNA probes are cell impermeant, requiring cell permeabilization. This is impractical in cell sorting, may lead to non-specific antibody labelling or unwanted cell loss and potentially RNA disruption. Therefore, DNA dyes such as PI, DAPI or 7-AAD are inappropriate. Alternatively, one can use the live-cell permeant DNA probe Hoechst 33342 that is excited by UV light. However, DNA binding by Hoechst 33342 is highly time-dependent such that it could drift during the long times often required for cell sorting. There is also the risk of DNA damage due to UV exposure that may impact downstream PCR, most important in very low copy number applications.

HOW DOES DRAQ5™ HELP?

The live-cell permeant DNA probe DRAQ5™ can be applied directly to complex samples such as tissue digests, blood or bone marrow rapidly labelling the nucleated cells, as the last step before sorting, without washing. At its simplest, individual G_0/G_1 (2N) cells can be gated from others within the cell cycle and from sub-$G_{0/1}$ cells (and debris) which maybe apoptotic and almost certainly have a different RNA expression profile. Nucleated cells throughout the cell cycle can be sorted based on DRAQ5™'s stable, stoichiometric labelling of dsDNA. Doublets are discarded ("dump" sorted) utilising 'area' versus 'width' data for the same DRAQ5™ cell signal. Dead cells are excluded by combining DRAQ5 with DAPI, for example.

Far-red fluorescence makes DRAQ5™ spectrally compatible with most visible-range chromophores for multi-colour cell sorting. DRAQ5™ is excited by blue or red laser lines, found on most modern cell sorters.

The resulting populations of sorted cells can be processed for PCR, RT-PCR or mass spectrometry. DRAQ5™ does not affect the performance of reverse transcriptase or taq polymerase. Note: cells treated with cell-permeant DNA intercalating agents are not suited to downstream culture or expansion, particularly at the concentrations required to deliver high quality DNA profiles.

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

DRAQ5™ Product Features:

- far-red fluorescing live-cell permeant dsDNA probe
- rapid, stable, stoichiometric labelling of all nucleated cells
- compatible with BV / BUV, FITC & R-PE dyes
- easy-to-use, direct from the fridge
- excited by red or blue laser lines

BioStatus Limited
56A Charnwood Road, Shepshed, Leicestershire LE12 9NP, United Kingdom
T +44 1509 558 163 | E enquiry@biostatus.com | W www.biostatus.com