DRAQ5™ in Cell Sorting (FACS)

Far-Red Fluorescent Live-Cell Permeant DNA Dye

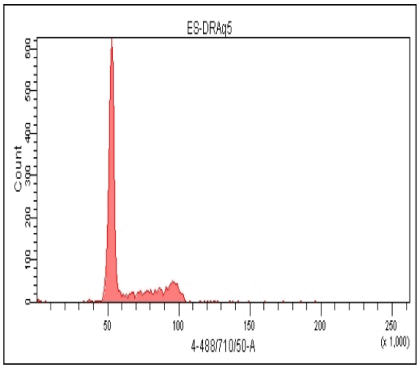
biostatus

DR5.APPNOTE: CELL SORTING 001 040814

1. CELL SORTING FOR PCR, BASED ON DIFFERENTIAL DNA CONTENT

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. The quantity of DNA in each nucleated cell in a population can be measured by labeling the nuclear DNA with a fluorescing DNA dye. The signals are plotted to create a DNA profile for the population giving information on the proliferative status, usually alongside phenotyping, that can be used for cell sorting and downstream for PCR or RT-PCR, thereby allowing study of gene mutation or copy number in the context of the cell cycle position of the portion of cells within the sorting parameters selected.



WHAT IS THE PROBLEM?

The majority of DNA probes are cell impermeant, requiring cell permeabilization. Doing this in cell sorting is impractical, may lead to non-specific labeling by antibodies or unwanted cell losses and certainly RNA disruption. Therefore, DNA dyes such as propidium iodide, 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, the DNA binding of Hoechst 33342 is highly time-dependent meaning that it could drift during the long times required for cell sorting. There is also the risk of DNA damage due to UV light exposure that may impact downstream PCR, most important in very low copy number applications.

HOW DOES DRAQ5™ HELP?

As a live-cell permeant DNA probe DRAQ5™ can be applied directly to complex samples such as tissue digests, blood or bone marrow leukocytes, rapidly labelling the nucleated cells, as the last step before sorting and without washing. Nucleated cells in different regions of the cell cycle are sorted on the basis of their DRAQ5 signal, due to its stable, stoichiometric labelling of dsDNA. Doublets may be “dump” sorted utilising the area versus width data for the same DRAQ5 cell signals.

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 and RT-PCR. 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
- 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

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