DRAQ5™ in Plate-Based Cytometry

Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: PLATE CYTOMETRY 002 060323

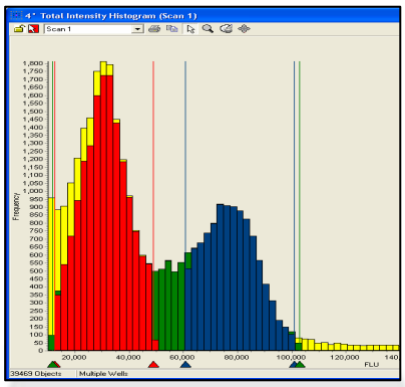
1. NUCLEATED CELL LABELLING & NUCLEAR SEGMENTATION

BACKGROUND

Microplate- and slide-based cytometers (e.g. TTP Labtech's Acumen eX^3 and Nexcelom's Celigo S) utilising laser scanning combine the morphological (textural) capability of fluorescence-based imaging with the cell-by-cell multi-parameter analysis of a cytometer. These instruments are particularly valuable for adherent cell types that are not readily compatible with flow cytometry. However, diluted whole peripheral blood samples have been analysed with such instruments for complex white and red cell analysis.

WHAT IS THE PROBLEM?

As with fluorescence microscopy individual cells can be interrogated and counted, with the possibility to segment the cells to their nuclear and cytoplasmic compartments. However, such identification requires addition of a fluorescent dsDNA counterstain. The ideal DNA counterstain to segment nuclei should meet the following criteria: discrete nuclear staining; spectrally separated from commonly used chromophores; work in live or fixed cells; report DNA content; cross-platform compatible for upstream assay development.



Whole blood analysis on slide-based cytometer requires a simple means of differentiating the nucleated and enucleated cells. This could be easily achieved with a DNA counterstain with the characteristics above.

HOW DOES DRAQ5™ HELP?

The far-red fluorescing cell-permeant DNA-binding probe DRAQ5™ discretely segments nucleus from cytoplasm in microplate-/slide-based cytometry in live or fixed cells, for detailed morphometric analyses. Binding to dsDNA is stoichiometric, enabling measurement of DNA content (see below) and morphological analysis of the nucleus (fragmentation, metaphase, etc.). DRAQ5™ can be combined with most UV-/violet-excited and visible-range chromophores for multi-colour experiments making it cross-platform compatible.

DRAQ5™ is cell permeant. It can be added at the end of a staining procedure for live or fixed cell assays. Likewise, DRAQ5™ is compatible with complex samples such as blood / bone marrow to label the nucleated cells, without resorting to RBC lysis. This allows these to be easily differentiated from the RBCs.

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

2. 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 labeling 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 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 a cell permeant DNA probe Hoechst 33342 excited by UV light. However, UV sources are less common on modern microplate-/slide-based cytometers being replaced by violet ones due to the emergence of new classes of violet–excited antibody labels.

HOW DOES DRAQ5™ HELP?

As a cell permeant DNA probe DRAQ5™ can be applied directly to adherent cultured cells or complex samples such as blood or bone marrow (as described above), as the last step prior to 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. It is possible to get DNA profiles with the minimum disturbance to the sample, especially important for precious, small samples or where there's risk of uncontrolled cell losses. Far-red fluorescence makes DRAQ5™ spectrally compatible with most visible range chromophores for multi-colour analysis. It is excited optimally by red laser lines, found on most cytometers. For convenience DRAQ5™ is supplied in an aqueous ready-to-use formulation, highly compatible with high throughput automation in drug discovery.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- water-soluble; easy-to-use from the fridge
- rapid, stoichiometric labelling of all nucleated cells
- compatible with visible-range fluorophores
- excited by yellow (561nm) to red (647nm) wavelengths