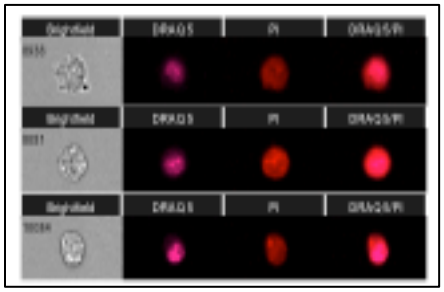
DRAQ5™ in Imaging Flow Cytometry

Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: IMAGING FCM 002 040814

1. NUCLEATED CELL LABELLING & NUCLEAR SEGMENTATION

BACKGROUND



Imaging flow cytometers (Amnis Imagestream and Flowsight) combine cell-by-cell multi-parameter analysis of a flow cytometer with morphological or textural analysis from a fluorescence microscope. A key feature is the ability to cross-compare phenotypically distinct cell types with morphological characteristics and so-called "similarity" analyses. It is possible to classify individual cell events from a dot-plot to further confirm their identities using bright field and individual fluorescence images and composites.

It is possible to analyse any sample suitable for an equivalent conventional flow cytometer, including blood and bone marrow samples that, of course, contain a mixture of nucleated and enucleated cells. When analysing the nucleated cells it is common to lyse the enucleated cells with NH_4Cl or similar. This is mostly preferable since the event analysis rate is somewhat lower than a conventional flow cytometer.

WHAT IS THE PROBLEM?

As in fluorescence microscopy one is able to view individual cells. One can potentially segment the cells to the nucleus and cytoplasmic compartment. However, this requires the addition of a fluorescent DNA counterstain. The ideal DNA counterstain to segment nuclei should meet all of the following criteria: show discrete nuclear staining; be spectrally separated from commonly used chromophores; work in live or fixed cells; report DNA content; be cross-platform compatible for higher resolution or routine applications.

Despite the relatively slow event analysis rate it may often be inappropriate to perform red cell lysis (RBC) lysis on blood / bone marrow. Examples of this include study of small, precious samples, looking for rare events or when analysing erythropoietic / thrombopoietic pathways or leukocyte-platelet aggregation.

HOW DOES DRAQ5™ HELP?

The far-red fluorescing cell-permeant DNA-binding probe DRAQ5™ discretely segments the nucleus from the cytoplasm in imaging flow cytometry in live or fixed cell experiments, for detailed morphometric analyses. Binding to DNA is stoichiometric also allowing measurement of DNA content. It 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 to a complex sample such as blood / bone marrow to label the nucleated cells. This allows these to be identified from enucleated cells without having to resort to time-consuming and risky RBC lysis.

DRAQ5™ Product Features:

- far-red fluorescing 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:
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