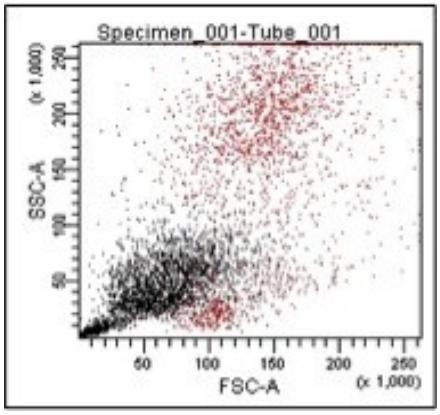
BIOSTATUS

CyTRAK Orange™ in Flow Cytometry

Orange Fluorescent Live-Cell Permeant DNA and Cytoplasmic Dye

1. NUCLEATED CELL GATING

BACKGROUND



Normal blood and bone marrow samples contain a mixture of nucleated cells (leukocytes - including lymphocytes, monocytes, neutrophils - and various progenitor cells) and enucleated cells (mature erythrocytes and platelets). Typically, there are around 40 platelets and 400 erythrocytes for every nucleated cell. These can interfere with analyses of the nucleated cells, especially in flow cytometry where they complicate and slow phenotypic analysis. The most common solution is to osmotically shock the enucleated cells with NH_4Cl – known as RBC lysis. After this, the nucleated cells are pelleted by centrifugation, washed, counted and resuspended for use.

WHAT IS THE PROBLEM?

There are many potential risks from RBC lysis: additional time required; release of debris into the sample that can aggregate with leukocytes; inconsistent results; possible lysis of erythroid precursors; non-specific cell losses during washing procedures. The relative importance of these may vary but obviously sample loss would be of concern with mouse tail vein samples or in the detection of extremely rare cells such as circulating tumor cells, for example.

HOW DOES CyTRAK Orange™ HELP?

The presence of genomic DNA is a simple way to differentiate between nucleated and enucleated cells. CyTRAK Orange™ is a live-cell permeant dsDNA-specific probe that efficiently and stably labels nucleated cells. It is added to diluted whole blood or bone marrow, mixed and briefly incubated. CyTRAK Orange™ fluoresces in the orange/red when excited by blue or green laser on standard flow cytometer. The signal is detected in a channel centred on 610 nm. This signal is then used to select exclusively or “gate” the nucleated cells without the complexity and risk associated with RBC lysis. The nucleated cell population may include rare “occult” tumor cells or endothelial cells not present in healthy blood or marrow. These are preserved by avoiding RBC lysis.

The orange/red fluorescence of CyTRAK Orange™ means that it can be combined with UV- and violet-excited chromophores, FITC / GFP and also with red-excited chromophores (e.g. APC, APC-conjugates) since CyTRAK Orange™ is not co-excited by red wavelengths.

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

2. MONITORING CELL SENESCENCE (EMERGING APPLICATION)

CyTRAK Orange™ can be used in flow cytometry to indicate that a population of cells is arresting or in senescence, typically seen when long-term cell cultures (e.g. for protein production) become exhausted. Such cells swell, becoming larger than the healthy cells. These larger cells take up more CyTRAK Orange™ and this can be seen in a flow cytometry intensity plot. Using this information one can make decisions when to establish a new culture. Contact BioStatus to discuss this application.

CyTRAK Orange™ Product Features:

- orange fluorescing cell permeant dsDNA probe
- rapidly and clearly labels all nucleated cells (live or fixed)
- single-channel dual compartment (nucl:cyto) segmentation
- compatible with Horizon BV / BUV, FITC & red-excited dyes
- water-soluble; ready-to-use from the fridge

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