CyTRAK Orange™ in HTFC Screening

Orange Fluorescent Live-Cell Permeant DNA and Cytoplasmic Dye

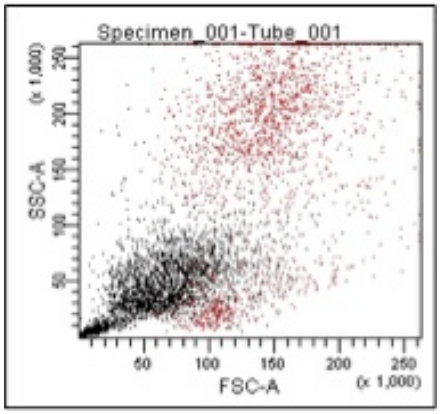
biostatus

CO5.APPNOTE: FLOW SCREEN 001 050814

1. NUCLEATED CELL GATING

BACKGROUND

Normal blood and bone marrow samples contain a mixture of nucleated cells and enucleated cells (mature erythrocytes i.e. RBCs and platelets). The RBCs can interfere with analysis 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.



Similarly, with cultured cells it is useful to be able to gate out debris that can range from subcellular fragments to whole cells with fragmenting nuclei.

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 cell 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.

It is difficult to set a gate to exclude any non-viable cells with fragmenting nuclei from intact viable cells. These non-viable cells may interfere with the analysis, particularly of rare cells.

HOW DOES CyTRAK Orange™ HELP?

The presence of nuclear DNA allows differentiation between nucleated and enucleated cells. CyTRAK Orange™ is a live-cell permeant dsDNA-binding 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 light on a standard flow cytometer. The signal is detected in a channel centred on 610 nm. (Usefully, it is not excited by red wavelengths). This signal is used to select or "gate" nucleated cells without the complexity and risk associated with RBC lysis. Nucleated cells may include rare tumor or endothelial cells not present in healthy blood or marrow, left untouched when processing is limited to a simple dilution step.

An increased CyTRAK Orange™ signal can identify cells that have experienced cell cycle arrest or undergoing senescence.

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.

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

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

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