

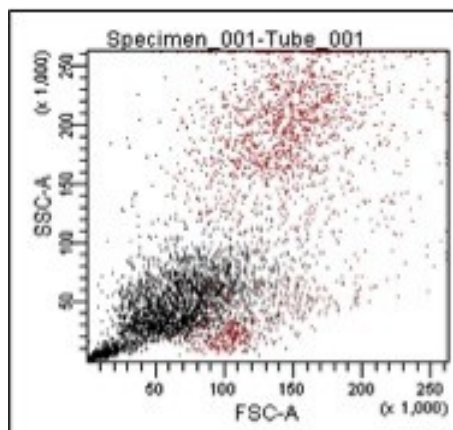
CyTRAK Orange™ in FC-Based *In Vitro* Tox

Orange Fluorescent Live-Cell Permeant DNA and Cytoplasmic Dye

1. NUCLEATED CELL GATING

BACKGROUND

In vitro toxicology testing on blood and bone marrow is an emerging application (hematotoxicity) in drug safety, aided by the developments in reagents and flow cytometry technologies. These samples contain a mixture of nucleated cells and enucleated cells and 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 NH₄Cl – 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 (causing a skewing of myeloid:erythroid ratios); non-specific cell losses during washing procedures. The relative importance of these may vary but obviously these can impact the performance of robust assays and use of automation.

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 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.

For convenience CyTRAK Orange™ is supplied in an aqueous ready-to-use formulation and is amenable to routine applications and automated procedures.

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



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