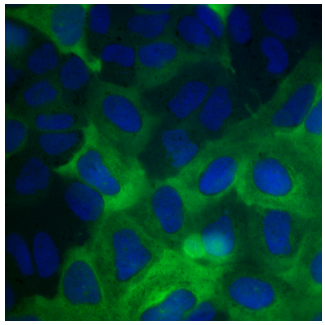
CyGEL™ in Cell Sorting

Thermoreversible Hydrogel Mountant for Live FACS Sorted Cells

CY1.APPNOTE: CELL SORTING 001 060815

1. MOUNTING VIABLE PHENOTYPICALLY-SORTED CELLS

BACKGROUND



Fluorescence Activated Cell Sorting (FACS) enables the separation of different cell phenotypes in a complex sample such as blood, bone marrow or tissue digest. This means that cells can be collected for onward expansion and differentiation, molecular analysis or fluorescence microscopy. In the latter case they can be deposited directly to an optical surface such as a microscope slide in arrays according to the pre-determined sorting parameters. This allows cells to be investigated for "texture", distribution and co-localisation for example.

WHAT IS THE PROBLEM?

The problem arises when sorted cells are to be subsequently analysed by fluorescence microscopy. It is possible to centrifuge cells down onto an optical surface (e.g. "cytospin") that will, however, inevitably impact on the native 3-D morphology of the cells, rendering the cells to essentially 2-D. To overcome this and retain the 3-D structure, they can be sorted onto and anchored to slides using adherent coatings such as poly-lysine that are typically costly but are at least compatible with live cells. Nonetheless, the non-adhered portion of the cells will remain plastic and unsuitable for high resolution imaging. If cells are sorted into a simple aqueous medium the degree of Brownian motion would make composite images impossible, at the very least. The cells may not distribute evenly due to settling, rolling and clumping that may be made worse by the 'panning' effects of movements of the slide. Typically the cells will concentrate in the centre or at the perimeter of the well. All of these approaches make high performance microscopy difficult.

HOW DOES CYGEL™ HELP?

An alternative provided by CyGEL™ is to sort the cells into an immobilizing, optically compatible hydrogel which can then be deposited onto a slide, microtiter plate or chambered slide.

CyGEL™ allows observation of living, viable cells in their native 3-D morphology without concerns over movement. CyGEL™ works as a thermo-reversible hydrogel - *liquid when cold and a gel when warmed*. It is designed as a flexible general purpose immobilising medium for the capture and analysis of suspension cells in microplates and not as a long-term growth matrix. Upon warming beyond room temperature to 37 °C, a CyGEL™ preparation rapidly transitions to a gel that can be returned within seconds to a liquid by simple cooling. This allows the operator to generate simple protocols for the capture and analysis of cell populations. CyGEL™ can be doped with counterstains and is compatible with fluorescence and standard microscopy optics.

In this specific case of sorting cells, CyGEL™ can be pipetted as an array of "buttons" of the hydrogel on a slide that are then the addressable positions for each of the phenotypes to be deposited for imaging. Alternatively, CyGEL™ can be pipetted into the wells of an imaging quality microtiterplate or chambered slide where each well receives the sorted phenotypes. For the deposition of the cells into CyGEL™ the hydrogel is chilled to keep it liquefied and then warmed thereafter to gently achieve the thermo-reversible immobilisation and application of coverslip as appropriate. (Sinkora & Sinkorova. J Immunol 193.10 (2014): 5023-5032).

CyGEL™ Product Features:

- convenient immobilisation of intact, viable cells, objects and calibrating beads
- optically clear with low autofluorescence for visible range excitation
- controllable performance and rapid reversible transition from liquid to gel
- compatible with GFP and "in gel" fluorescent probes including DRAQ5™

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