CyGEL™ in Immunofluorescence

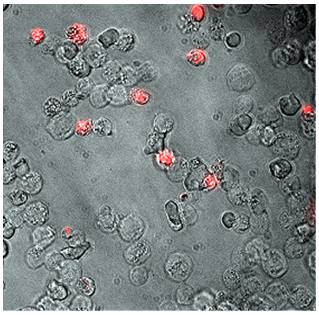
Thermoreversible Hydrogel Mountant for Non-adherent/Motile Cells

biostatus

CY1.APPNOTE: IMMUNOFLUOR 001 180814

1. MOUNTING NON-ADHERENT CELLS / FRAGILE OBJECTS

BACKGROUND



Immunofluorescence microscopy allows the detailed 2-D examination of adherent cell phenotypes and tissue sections that have been preserved by fixation with formaldehyde or similar. It is possible to combine 2 or 3 features each labelled with fluorescent antibodies, usually with the addition of a nuclear counterstain. The images can all be overlaid with the phase contrast or transmission image for further information since these objects are attached to the optical surface of slide or well. One can analyse individual cells or "neighbourhoods" of a tissue section repeatedly in a relatively time-independent manner and with different illuminations for each chromophores and be confident that it will be possible to overlay them on each other with good precision for a composite image. The use of this approach to study cells allows for analysis of internal "texture" that is not possible by flow cytometry.

WHAT IS THE PROBLEM?

The problem arises when non-adherent cells are to be analysed by fluorescence microscopy. It is possible to centrifuge cells down onto an optical surface (e.g. "cytospins") that will inevitably impact on the native 3-D morphology of the cells. They can be anchored using adherent coatings that are typically costly and may be more compatible with live cells. Otherwise, however, non-adherent cells will remain subject to Brownian motion if they are mounted in a simple aqueous buffer following staining procedures making preparation of composite images impossible. 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. Non-adherent cells will concentrate in the centre or at the perimeter of the well. All of these approaches potentially introduce bias in the cells that are immobilized.

Also, in the case of fragile tissue pieces these can be damaged by efforts to make them mount flat after already processing them for immunofluorescence labeling. It may be better to find a means of immobilizing them that encases such objects in their native configuration.

HOW DOES CYGEL™ HELP?

An alternative provided by CyGEL™ is to capture *all* cells after staining in an immobilizing, optically compatible hydrogel which can then be deposited onto a slide or chambered coverslip.

CyGEL™ allows observation of 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. The gel is formulated to be liquid when cool so can be conveniently stored at 4 °C and mixed with a cell preparation. 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.

CyGEL™ Product Features:

- convenient immobilisation of non-adherent 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:
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