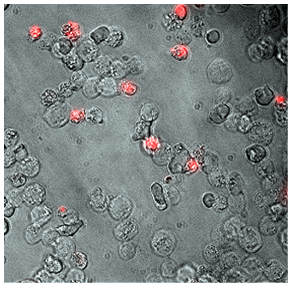
CyGEL™ in Live End-Point Imaging

Thermoreversible Hydrogel Mountant for Non-adherent/Motile Cells

CY1.APPNOTE: LIVE ENDPOINT 001 180814

1. MOUNTING LIVE NON-ADHERENT CELLS / FRAGILE OBJECTS

BACKGROUND



In fluorescence-based microscopy it is normal to preserve cells or tissue sections with fixative. This works by crosslinking proteins. There are circumstances where fixation is detrimental, for example, if the target leaks into other sub-cellular compartments or out of the cell on washing. Also, fixation may produce artefacts. Fixation is inappropriate in assays to study drug-receptor interactions as cells are imaged after test antagonists and again after the reference agonist(s). The same would be true in assays to follow internalization or processing of surface-bound molecule (e.g. GPCRs). One could envisage such assays where cells express a target of interest tagged with a fluorescent protein (e.g. GFP, DsRed) or labelled by one or more fluorescent cell-permeant functional probes. Imaging is chosen as it provides "textural" information on cells, unlike flow cytometry.

WHAT IS THE PROBLEM?

The problem arises when the cells in question are non-adherent or de-adhered. It is not possible to centrifuge cells down onto an optical surface since that will inevitably impact on the native 3-D morphology of the cells, at best. Cells can be anchored using adherent coatings that are typically costly and again may disturb the morphology. Otherwise, however, non-adherent cells will remain subject to Brownian motion and they may be actively motile if they are mounted in a simple culture medium 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 often concentrate in the centre or at the perimeter of the well. All of these approaches potentially introduce bias in the cells that are immobilized.

HOW DOES CyGEL™ HELP?

An alternative is to capture *all* cells in an immobilizing, optically compatible hydrogel that can be deposited onto a slide - CyGEL™ allows you to observe live cells in real-time experiments without motility or movement. CyGEL™ is thermoreversible - *liquid when cold and a gel when warmed* – and compatible with LIVE cells suspensions. It is designed as a flexible immobilising medium for capture and analysis of suspension cells and not as a growth matrix. It is formulated to be liquid when cool so can be stored at 4 °C and mixed with a cell preparation. Upon warming beyond 22 °C to 37 °C, CyGEL™ rapidly transitions to a gel that can be liquefied within seconds by simple cooling. It can also be gently dissolved by adding excess chilled buffer. This allows one to generate simple protocols for capture, analysis and, indeed, recovery of cell populations. CyGEL™ is compatible with culture media, small molecules (viability and vital dyes, anaesthetics), fluorescence and standard microscopy optics.

CyGEL™ allows you to explore different imaging applications including incorporating evenly distributed reference beads into your samples for simple controls or 'in sample' calibrations. You can exploit the fact that cells within a hydrogel experience reduced diffusion rates for permeant molecules across the plasma membrane - both into and out of the cell. For example, viable cell DNA dyes such as DRAQ5™ when incorporated into CyGEL™ enter cells at reduced diffusion controlled rates, stretching the difference between rapidly-staining dead cells and slow-staining viable cells. Alternatively, a gel 'overlay' for adherent cells in a micro-well will reduce the loss of reporter molecules – extending the analysis window for processing in a live cell imaging assay.

CyGEL™ Product Features:

- convenient immobilisation of non-adherent cells and 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