CyGEL Sustain™ in Image-Based Screening

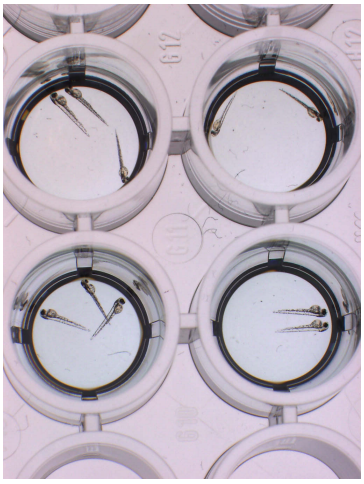
Long-Acting Thermoreversible Hydrogel Mountant for Non-adherent/Motile Cells

biostatus

CS2.APPNOTE: IMAGE SCREEN 001 200814

1. IMMOBILISING NON-ADHERENT CELLS/MICRO-TISSUES IN MICROPLATES

BACKGROUND



In cell-/tissue-based assays for high content screening (HCS, target-based or phenotypic) fluorescence-based imaging is the preferred approach when it is important to understand changes that occur in the context of morphology or of intracellular texture. This allows a number of parameters to be studied simultaneously: a disease-related readout such as a protein translocation, changes in cell numbers and morphology (sentinels for toxicity), a functional one such as mitochondrial health, cell:cell interactions or even organotypic relevance in the case of micro-tissues or model organisms.

In principle these assays can be performed on live adherent, non-adherent / suspension cells, micro-tissues and model organisms such as zebrafish.

WHAT IS THE PROBLEM?

The problem arises when non-adherent or motile objects need to be used in microplate formats for imaging since they are essentially mobile within the well. Non-adherent objects in a conventional culture medium or buffer often do not distribute evenly within the micro-well due to settling, rolling and clumping artifacts made worse by the 'panning' effects of movements of the microplate. Frequently non-adherent cells and motile organisms will concentrate in the centre or at the perimeter of the well. To overcome these issues suspension cells can be centrifuged onto the well surface and fixed or 'tacked' to the surface using adhesion reagents – all potentially introducing bias in the cells that are immobilized. Centrifugation may not be compatible with fragile micro-tissues and may not properly orientate model organisms. Additionally, certain live objects may require specific nutrient and tonicity conditions even during imaging. One example of this might be zebrafish embryos (embryo/river water) or epithelial cells (DMEM F12, EGF, Insulin).

HOW DOES CyGEL Sustain™ HELP?

An alternative provided by CyGEL Sustain™ is to immobilize these objects in an optically compatible hydrogel. CyGEL Sustain™ allows you to observe live objects for multiple illuminations, z-sections for 3D rendering or to re-visit a location without worrying about their rapid motility or movement in wells or on slides. It is not intended as a long-term growth matrix, however it is designed for the addition of culture medium specific to the cells or organisms under investigation. CyGEL Sustain™ works as a thermoreversible hydrogel - *liquid when cold and a gel when warmed*. It is compatible with LIVE cells suspensions, micro-tissues and model organisms. The gel is formulated to be liquid when cool so can be conveniently stored at 4 °C and mixed with a cell preparation, or ready in wells for delivery of objects or used as an overlay in slides or wells. Upon warming beyond 22 °C to 37 °C, a CyGEL Sustain™ preparation rapidly transitions to a gel. This allows the operator to generate simple protocols for the capture and analysis of non-adherent or motile objects. CyGEL Sustain™ is compatible with small molecules such as viability dyes, vital dyes and anaesthetics, fluorescence and standard microscopy optics. CyGEL Sustain™ does not disturb the imaging of fluorescent proteins. If required, CyGEL Sustain™ uniquely allows non-destructive recovery of live objects embedded in it.

CyGEL Sustain™ 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 DRAQ7™

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