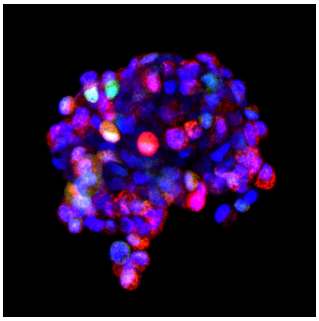
CyGEL™ in Image-Based *In Vitro* Tox

Thermoreversible Hydrogel Mountant for Non-adherent/Motile Cells

CY1.APPNOTE: IMAGE IVT 001 200814

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

BACKGROUND



One of the biggest costs in drug discovery and development has been failure of drug candidates late in the process (or after launch) due to unexpected or idiosyncratic toxicity and risk to patient health. To reduce this compound libraries are exposed to a battery of mandatory tests, however these are performed when the new chemical entity (NCE) is often well advanced. Recently, in vitro toxicology (IVT) assays have been developed that use physiologically relevant cells, micro-tissues or model organisms to measure their health at different NCE concentrations using a few simple parameters from, for example, total cell count, plasma membrane failure, mitochondrial failure or reactive oxygen species (ROS) presence. Changes in these parameters are detected using fluorescent functional probes.

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.

HOW DOES CyGEL™ HELP?

An alternative provided by CyGEL™ is to immobilize these objects in an optically compatible hydrogel. CyGEL™ 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. CyGEL™ 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™ 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™ (PBS-formulated) is compatible with culture media, small molecules such as viability dyes, vital dyes and anaesthetics, fluorescence and standard microscopy optics. CyGEL™ does not disturb the imaging of fluorescent proteins. If required, CyGEL™ uniquely allows non-destructive recovery of live objects embedded in it.

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:
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