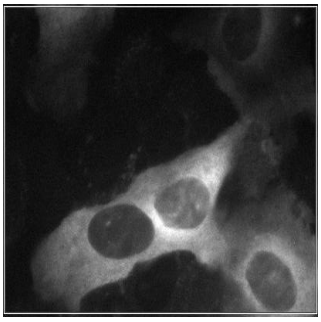
BIOSTATUS

CyGEL Sustain™ in Plate-Based Cytometry

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

1. IMMOBILISING NON-ADHERENT CELLS IN MICROPLATES

BACKGROUND



For some applications flow cytometry has shortcomings, including a relatively low throughput and frequent requirement for large numbers of cells. A fundamental problem is the inability to analyze adherent cell lines *in situ* or 'revisit' the same cell or even 'neighbourhood' of a cell population. Image-based microplate cytometry offers a solution with a typical reduction in sample volume and an increase in the number of samples that can be analysed conveniently. The nature of a microplate format allows an instrument to revisit the same well address or a known location within a well.

WHAT IS THE PROBLEM?

The microplate format allows an instrument to revisit the same well address or even a known location within a well. The problem arises when the objects of interest move due to manipulation of the plate, movement of liquid in the well, disturbance due to plate manipulation, settling or indeed floating of the objects. The causes problems for image capture, event analysis and time-based studies. Sometimes there is a need to immobilise cells or even whole organisms over several hours to analyse events or signals of interest. Organisms capable of independent movement present a significant challenge in this regard. The problem is highlighted by the increasing use of zebrafish, *Danio rerio*, as a relevant model for vertebrate development. In short the problem is how to immobilize such organisms so that the analysis is not confused by independent movement. Under some circumstances limiting the degree of whole organism movement can permit more robust analysis of organ behavior, for example eye movement. The CyGEL Sustain™ thermoreversible hydrogel formulation is designed to offer a solution to these problems.

HOW DOES DRAQ7 HELP?

CyGEL Sustain™ allows you to observe live cells/organisms in real-time experiments without worrying about their motility or movement. CyGEL Sustain™ is a novel formulation of the thermoreversible CyGEL™. It shares the properties of CyGEL™ in that it is a liquid when cold and a gel when warmed! However, the CyGEL Sustain™ formulation is designed to extend the imaging period for LIVE Zebrafish embryos for several hours while maintaining whole organism immobilization. CyGEL Sustain™ can also extend the imaging period for live cell preparations - depending on cell/organism type. Its special formulation is optimised for the addition of RPMI and similar culture media. For live C.*elegans* and other parasite imaging we recommend CyGEL™.

The gel is formulated to receive culture medium (e.g. RPMI) and to perform as a liquid when cool so can be conveniently pre-stored at 4 °C. Upon warming beyond room temperature a CyGEL Sustain™ preparation rapidly transitions to a gel immobilizing the organism in the well. The preparation can be returned within seconds to a liquid by simple cooling. CyGEL™ gel/organism preparations can be dissolved by the addition of chilled excess buffer, releasing the organism for further study or processing. This allows the operator to generate simple protocols for capture, analysis and indeed recovery of objects of interest. CyGEL Sustain™ is compatible with viability and vital dyes, anaesthetics and other small molecules, fluorescence and standard microscopy optics.

CyGEL Sustain™ Product Features:

- convenient immobilisation of non-adherent cells, beads and organisms
- optically clear with low autofluorescence for visible range excitation
- permits long term analysis of live non-adherent cells (non-growth matrix)
- permits sterile, low temperature recovery of cells and organisms from gel
- compatible with GFP and "in gel" fluorescent probes including DRAQ5™
- multiple assay formats and applications including apoptosis detection

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