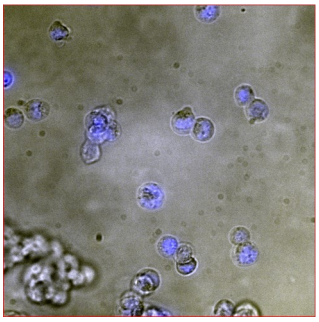
BIOSTATUS

CyGEL Sustain™ in Image-Based Apoptosis Assays

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

1. EXTENDED IMMOBILISATION OF NON-ADHERENT / DE-ADHERING CELLS

BACKGROUND



Microscopy offers the ability to gain important morphological information, including nuclear appearance to identify apoptotic events, or even subcellular location of other markers of interest. Cells undergoing death processes modify both localization and mobility properties of DNA-binding proteins and other compartmentalised and membrane-located proteins allowing stages of apoptosis to be resolved. Sometimes there is a need to immobilise cells or even whole organisms over several hours to analyse events or signals of interest.

WHAT IS THE PROBLEM?

For apoptosis detection by microscopy ease of sample analysis often depends on time course of cell death and whether cells under study are adherent or in suspension. Adherent cells may be lost due to death in mitosis or loss of adherence. Suspension cultures are subject to loss of apoptotic cells if a protocol requires cell washing / centrifugation for additional marker analysis. The ability to immobilise cells either on a surface or within a 3-D matrix is essential for microscopy-based apoptosis detection and event counting.

Problems arise in microscopy when the analysis time period needs to be extended and yet still have the ability to track events at specific locations over time. Use of a cell impermeant DNA probe, doped into a 3D immobilising matrix, to monitor plasma membrane integrity would be highly advantageous. However, such a reagent must not enter intact cells (i.e. be truly non-toxic), only permeating cells with compromised membranes and spectrally compatible with other agents to be used in the assay system.

HOW DOES CyGEL Sustain™ HELP?

CyGEL Sustain™ provides an optimised solution for cell immobilisation, compatible with LIVE cells suspensions. CyGEL Sustain™ is a novel formulation of thermo-reversible CyGEL™, sharing its properties - liquid when cold and a gel when warmed! CyGEL Sustain™ allows you to observe live cells/organisms in real-time experiments without worrying about their movement. CyGEL Sustain™ can extend the imaging period for live cell preparations - depending on cell/organism type. Its special formulation is optimised for addition of RPMI and similar culture media. CyGEL Sustain™ is formulated to receive culture medium (eg RPMI) and perform as a liquid when cool so can be conveniently pre-stored at 4 °C. Upon warming above 22 °C a CyGEL Sustain™ preparation rapidly transitions to a gel immobilizing organism(s) in the well. The preparation can be returned within seconds to a liquid by simple cooling. CyGEL™ / organism preparations can be irreversibly dissolved by addition of chilled excess buffer, releasing organisms 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 fluorescence and standard microscopy optics. Immobilising cells with CyGel Sustain™ is a convenient method for analysing live and dead cell populations – generated from adherent cultures or suspension cultures. The far-red DNA binding viability probe DRAQ7™ can be incorporated into CyGEL™ to provide *in situ* staining of cells for time-resolved tracking of cell status (due to validated non-toxicity) or as pre-stain for cells prior to immobilisation.

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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