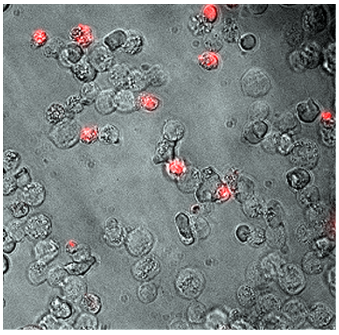
CyGEL™ in Image-Based Apoptosis Assays

Thermoreversible Hydrogel Mountant for Non-adherent/Motile Cells

CY1.APPNOTE: IMAGE APOPTOSIS 001 290714

1. IMMOBILISING NON-ADHERENT AND DE-ADHERING CELLS

BACKGROUND



Microscopy offers the ability to gain important morphological information, including nuclear appearance to identify apoptotic events, or even the 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. Cell immobilisation potentially offers advantages for the analysis of cell death in complex populations.

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. Problems also arise when the fluorescence signal intensity of apoptotic cells is variable – a feature of many DNA staining dyes due to the complex matrix of cell status, dye permeation properties, dye binding specificity, dye binding modes and dye-dye interactions. However the spectral properties of impermeant dyes for detecting cell death often determine their practical application with other reporters. DNA specific stains are preferred although choice is dependent upon the wavelengths of light available for probe excitation. The staining of chromatin in such cells, with varying levels of structural integrity, by both permeant and non-permeant dyes, is complex and not readily predicted. Hyperchromatic staining of apoptotic nuclei with absorption cationic dyes is frequently observed while apoptotic cell nuclei often appear dim with many DNA fluorochromes. The enhanced affinity of the chromatin of early apoptotic cells for cationic dyes is associated with conformational relaxation rather than degradation of DNA. In late apoptotic cells, the dense packaging of degraded DNA promotes further aggregation of dyes affecting properties and appearance of nuclei. Being able to identify these different stages in non-adherent cells presents a challenge for microscopy.

HOW DOES CyGEL™ HELP?

CyGEL™ works as a thermoreversible hydrogel - liquid when cold and a gel when warmed. It is compatible with LIVE cells suspensions. It is designed as a flexible general purpose immobilising medium for the capture and analysis of suspension cell populations in microplates and not as a long-term growth matrix. The gel is formulated to be liquid when cool so can be conveniently stored at 4 °C and mixed with a cell preparation. Upon warming beyond 22 °C to 37 °C, a CyGEL™ preparation rapidly transitions to a gel that can be returned within seconds to a liquid by simple cooling. Immobilising cells with CyGEL™ is a convenient method for analysing live and dead cell populations – generated from adherent cultures or suspension cultures. DRAQ5™ and DRAQ7™ fluorescent DNA dyes can be incorporated into CyGEL™ to provide in situ staining of cells or used as pre-stains for cell samples prior to immobilisation. An interesting feature of DRAQ5™ and DRAQ7™ dyes is the intense blue colour imparted to cells successfully stained – these cells can be readily seen in the cell pellets of suspension cells prepared by centrifugation – providing a visual check on the efficiency of cell harvesting if this is used prior to preparation with CyGEL™.

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