CyGEL™ in Live Organism Imaging

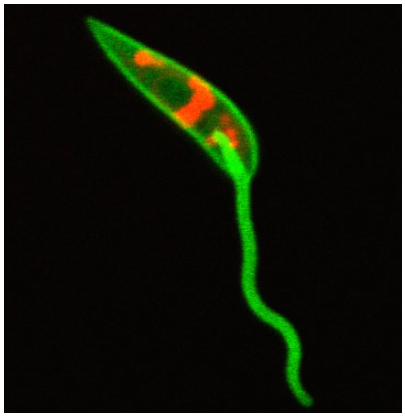
Thermoreversible Hydrogel Mountant (PBS) for Motile Organisms

CY1.APPNOTE: LIVE ORGANISM 001 190814

1. REVERSIBLE IMMOBILIZATION OF LIVE MOTILE ORGANISMS

BACKGROUND

Model organisms and parasites have become a important component of research giving access to useful surrogates of human health an disease, adding understanding to complex processes developmental biology and combining species-specific biology with genomic information. Likewise, technical advances in cell culture and stem cell biology have led to methods utilising 3D micro-tissues including spheroids and histoids.



Amongst the techniques available, high-performance microscopy is a powerful tool when combined with fluorescent proteins, antibodies and functional probes which have the potential to track complex events in live organisms and multicellular structures in real time.

WHAT IS THE PROBLEM?

Organisms such as tropical parasites (e.g. leishmania, trypanosomes), helminths (e.g. *C. elegans*) and drosophila embryos are all motile. Meanwhile, 3D micro-tissues are non-adherent and often fragile. In both cases, immobilization is required for high-resolution microscopy but mounting these in a non-destructive manner is extremely challenging. One approach would be to anchor the organism or microtissue to an adherent surface coating on a slide either by passive sedimentation or forced by slow-speed centrifugation. One is unable to control the orientation of the organism and this approach will probably change the morphology of a 3D micro-tissue. One alternative has been methylcellulose as a high viscosity liquid to eliminate some movement but suffering from unwanted opacity. Low-melting point agarose has been attempted but its melting point is such that it solidifies at temperatures above those physiological for the organism. In all cases, these methods make it difficult to correctly position the object for imaging. Similarly, intact and viable recovery for onward growth or observation is challenging if not impossible in all cases. An intact structure, albeit non-viable after imaging, would be required for suitable dissection prior to PCR or protein analysis.

HOW DOES CyGEL™ HELP?

An alternative is to capture or overlay the object(s) in an immobilizing, optically compatible hydrogel that can be deposited onto a slide or well - CyGEL™ - that allows you to observe live organisms and 3D micro-tissues in real-time experiments without motility or movement. CyGEL™ is thermoreversible – a *liquid when cold and a gel when warmed*. It is designed as a flexible immobilising medium for capture and imaging and not as a growth matrix. It is formulated to be liquid when cool so can be stored at 4 °C. Upon warming beyond 22 °C to 37 °C, CyGEL™ rapidly transitions to a gel that can be re-liquefied within seconds by simple cooling. It can be gently dissolved by adding excess chilled buffer for recovery of the objects embedded in it for onward growth or orthogonal analysis such as dissection, (RT-)PCR or protein extraction. This allows one to generate simple protocols for capture, analysis and, indeed, recovery of organisms. CyGEL™, formulated with PBS, is compatible with culture media, small molecules (viability and vital dyes, anaesthetics which may reduce twitching), fluorescence and standard microscopy optics.

CyGEL™ Product Features:

- convenient immobilisation of motile organisms and micro-tissues
- 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:
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