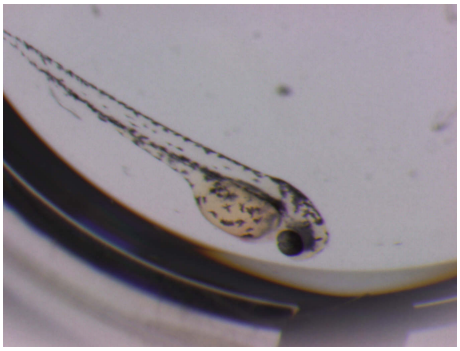
CyGEL Sustain™ in Live Organism Imaging

Medium-Ready Thermoreversible Hydrogel Mountant for Motile Organisms

CS2.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 *Danio rerio* embryos are all motile. 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 to an adherent surface coating on a slide either by passive sedimentation or forced by slow-speed centrifugation but this does not allow control of orientation of the organism. A possible alternative has been methylcellulose as a high viscosity liquid to eliminate some movement but suffers from unwanted opacity. Low-melting point agarose has been attempted but its melting point is such that it solidifies at temperatures above that physiological for most multicellular organisms. In all cases, these methods make it difficult to correctly position the organism 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. None of these allows preparation with the culture medium of choice for the organism under investigation, which may be a pre-requisite for longer-term imaging.

HOW DOES CyGEL Sustain™ HELP?

An alternative is to capture or overlay the organism in an immobilizing, optically compatible hydrogel that can be deposited onto a slide or well – CyGEL Sustain™ - that allows you to observe live organisms and 3D micro-tissues in real-time experiments without motility or movement. CyGEL Sustain™ is thermoreversible – a *liquid when cold and a gel when warmed*. It is designed as a flexible immobilising gel of optimal nutrient and tonicity for capture and extended 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 Sustain™ 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 organism(s) 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 Sustain™, is culture media-ready, and accepts small molecules (viability dyes, anaesthetics like MS-222), and is fully compatible with fluorescence and standard microscopy optics.

CyGEL Sustain™ 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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