CyGEL Sustain™ TECHNICAL DATA SHEET

PRODUCT: CyGEL Sustain™
PRODUCT NUMBERS: CS20500

PRESENTATION: aqueous solution.
STORAGE: store at 2-8 ºC. Do not freeze.

DESCRIPTION:
CyGEL Sustain™ is a novel thermo-reversible mountant that is compatible with extended imaging of live cells, tissues and organisms. It immobilizes non-adherent objects by simple warming and allowing their recovery by simple cooling. CyGEL Sustain™ is unusual in that it is a liquid when cold and a gel when warmed. CyGEL Sustain™ has ideal optical properties – low auto-fluorescence, R.I. ~1.37, clear, and non-quenching. CyGEL Sustain™ is specially formulated to be culture-medium ready for extended imaging applications: 4 to 6 hours, depending on cell type.

APPLICATIONS:
- advanced microscopy / CLEM:
  extended imaging live de-adhered / non-adherent cells (Upton, 2007)
  imaging live tissues and spheroids (Robertson, 2010)
  imaging and manipulating live *zebrafish embryos* (Alvarez, 2009)

BEFORE STARTING:
Read the MSDS. Wear protective clothing, safety goggles and laboratory gloves.
MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:
Culture medium (e.g. 10X RPMI, antibiotics, glutamine, sodium bicarbonate, sodium hydroxide), plasticware, ice-bath/-pack, DRAQ5™, DRAQ7™, propidium iodide.

EXAMPLE PROTOCOLS
PROTOCOL 1:
PREPARATION OF CyGEL Sustain™ FOR USE WITH RPMI 1640
As supplied, CyGEL Sustain™ will transit from sol to gel at 23-24°C.

1. Cool an unopened vial of CyGEL Sustain™ on ice for 1-2 minutes.
2. Using a sterile pipette tip, add 87.5 µl of RPMI culture medium (see table 1). Mix thoroughly, taking care to avoid bubble formation.

RPMI-primed CyGEL Sustain™ is now isotonically correct for addition of viable cells. It will now transit from sol to gel at 20-21°C, usually within 1-2 minutes.

NOTE: The total volume of cells, beads and dyes added to the RPMI-primed CyGEL Sustain™ should not exceed 20% v/v. Over-dilution will result in loss of ability to gel.

PROTOCOL 2:
CyGEL Sustain™ MOUNTING OF CELLS ONTO A STANDARD MICROSCOPE SLIDE

1. Cool an unused vial of RPMI-primed CyGEL Sustain™ on ice.
2. Prepare cells for mounting in CyGEL Sustain™: Wash the cells in buffer (e.g. PBS) by centrifugation. Resuspend the cell pellet in a maximum of 50 µl buffer (1 - 5 x 10^5 cells depending upon the application).
3. Pipette the cell suspension into the vial containing PBS-primed CyGEL Sustain™.
4. Transfer 250ul of CyGEL Sustain™/cell suspension into a cold P1000 pipette tip. Quickly dispense onto a clean slide by streaking along the surface for the length of coverslip to be applied (see fig. 1). Repeat the process for the remaining CyGEL Sustain™/cell suspension – giving two microscope slide preparations.
5. Apply a coverslip to each CyGEL Sustain™/cell suspension "button" (see fig. 2).
6. Place microscope slides onto an ice pack to allow the CyGEL Sustain™ to liquefy whereupon the sample will spread out under the coverslip. Remove the slide from the ice pack. The CyGEL Sustain™ will now re-set as it reaches room temperature. The sample is now ready for visualization.

CyGEL Sustain™
KEY CHARACTERISTICS:
- Liquid below 20/21°C and gel above
- Infinitely thermo-reversible
- Refractive index: ~1.37
- Optically clear and inert, without visible range auto-fluorescence
- Viscosity increases with temp., between 21 and 28°C and plateaus beyond 37°C
- Excipients modify the viscosity and the sol:gel transition temperature
- Recommended max. dilution: <20% v/v

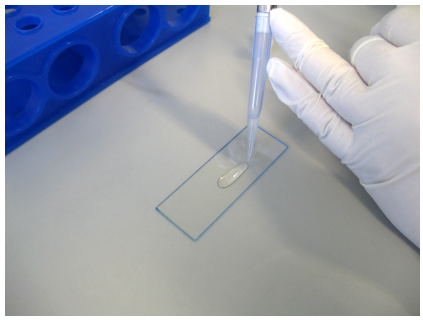

Fig. 1 Using a P1000 pipette tip streak cold CyGEL Sustain™ along the slide for the length of a coverslip

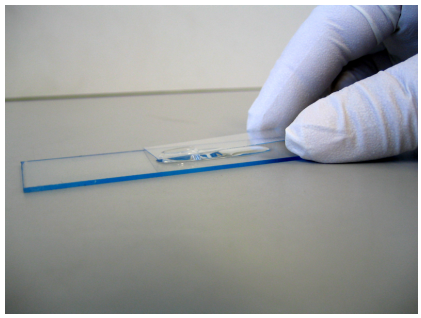

Fig. 2 Apply the coverslip from one end of the streak of CyGEL Sustain™ taking care not to trap bubbles. One applied, cool the slide on an ice-pack to allow the CyGEL Sustain™ to liquefy and spread under the coverslip.

Table 1.
Preparing RPMI culture medium for dedicated use with ***CyGEL™ Sustain***

To 20 ml 10X RPMI (Sigma:R1145), add:-
- 2.0 ml Penicillin(10^4 U) / Streptomycin (10mg/ml) in 0.85% NaCl (Invitrogen:15140-148)
- 2.0 ml L-Glutamine 200 mM; (Sigma:G7513)
- 5.4 ml 7.5% sodium bicarbonate soln. (Sigma:S8761)
- 0.4 ml 1N sodium hydroxide

For a final volume of RPMI-stock of 29.8 ml

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PROTOCOL 3:
CyGEL Sustain™ AS A DELIVERY MEDIUM FOR A CELL-PERMEANT DYE (DRAQ5™) IN FLUORESCENT IMAGING OF ADHERENT CELLS

1. Cool an unused vial of RPMI-primed CyGEL Sustain™.
2. Pipette 2.4 µl DRAQ5™ (5 mM stock) and dispense into the CyGEL Sustain™ and mix thoroughly. DRAQ5™ is now at a concentration of 20 µM, sufficient for stoichiometric chromatin binding.
3. For the addition of cells, continue by following Protocol 2 above.

DRAQ5™ nuclear staining should completely equilibrate after 60-80 min. However, sufficient staining should allow imaging of nuclei after 20-30 min. The individual nuclear fluorescence intensity with DRAQ5™ for each cell measured will reflect the cell cycle age distribution across the population.

PROTOCOL 4:
CyGEL Sustain™ AS A DELIVERY MEDIUM FOR A CELL-IMPERMEANT DYE (e.g. DRAQ7™) IN TIME-LAPSED FLUORESCENT IMAGING OF MEMBRANE-COMPROMISED CELLS

1. Cool an unopened vial of RPMI-primed CyGEL Sustain™.
2. Pipette 5.9 µl DRAQ7™ stock solution and dispense into the CyGEL Sustain™ and mix thoroughly. DRAQ7™ is now at a concentration of 3 µM, sufficient for reliable labeling of membrane-compromised cells.

   For the addition of cells, continue by following Protocol 2 above.
   Membrane-compromised (i.e. dying / apoptotic) cells will no longer be able to exclude DRAQ7™ and will appear fluorescent under excitation with the appropriate wavelength.

   For propidium iodide: from a 1mg/ml solution add 3 µl to an unused vial of primed CyGEL Sustain™.

PROTOCOL 5:
CyGEL Sustain™ MOUNTING OF CELLS IN A CHAMBER COVERSLIP FOR MICROSCOPY
1. Cool an unused vial of RPMI-primed CyGEL Sustain™ on ice / ice pack.
2. Prepare cell suspension: Wash the cells in buffer (e.g. PBS) by centrifugation.
3. Resuspend the cell pellet in the same buffer at a suggested concentration of 2 x 10^6 cells/ml.
4. Pipette 25 µl of the cell suspension into a clean chamber of a microscope 8-chamber coverslip.
5. Transfer 250 µl CyGEL Sustain™ with a P1000 pipette tip and directly overlay the cells in the chamber.
6. Warm the chamber above room temperature (e.g. on a thermally-controlled stage). The CyGEL Sustain™ layer will set thereby immobilizing cells and cell clusters for visualization.

It is possible to create wells for CyGEL Sustain™ using microscope slides and silicone o-rings (i.d. 8-10 mm). Smear Silicone (vacuum) grease onto an o-ring and bed down onto the slide to creates a well that can be filled with CyGEL Sustain™. If bedded onto a coverslip-bottomed petri-dish, the o-ring can be first surrounded with low-melting point agarose to create a moisture buffer for time-lapse imaging of live organisms (e.g. Danio embryos). Please contact BioStatus Technical Support to discuss your specific application of CyGEL Sustain™ for feasibility and technical tips.

KEY REFERENCES:
Upton, J-P., et al. (2007) Cell Death Different. 932-942
Edward, R., (2010) Am. Biotech. Lab. July/Aug: 12-14
Robertson, F.M., et al. (2010) J. Biomolec. Screen. 15: 820-829

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