CyGEL™ TECHNICAL DATA SHEET

PRODUCT: CyGEL™
PRODUCT NUMBERS: CY10500, CY20000
PRESENTATION: aqueous solution.
STORAGE: store at 2-8 ºC. Do not freeze.

DESCRIPTION:
CyGEL™ is a novel thermo-reversible mountant that is compatible with LIVE cells, tissues, organisms and beads. It can be used to immobilize non-adherent objects by simple warming and conversely allowing their recovery by simple cooling. CyGEL™ is unusual in that it is a liquid when cold and a gel when warmed. CyGEL™ has ideal optical properties – low auto-fluorescence, R.I. ~1.37, clear, and non-quenching.

APPLICATIONS:
- advanced microscopy / CLEM:
  imaging live de-adhered / non-adherent cells (Upton, 2007)
  imaging live and fixed tissues (Jecna, 2013) and spheroids (Robertson, 2010)
  imaging live *C. elegans*, fruitfly larvae (Chinta, 2012) and parasites (Price, 2009)
  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:
Buffers, culture medium, plasticware, ice-bath/-pack, DRAQ5™, DRAQ7™ and propidium iodide.

PROTOCOL 1:
PREPARATION OF CyGEL™ FOR USE
(As supplied, CyGEL™ will transit from sol to gel at ca. 23°C)

1. Cool an unopened vial of CyGEL™ on ice for 1-2 minutes.
2. Using a sterile pipette tip, add 12.8 µl of the supplied 40X PBS (i.e. 2.5% v/v). Mix thoroughly, taking care to avoid bubble formation.

The PBS-primed CyGEL™ is now isotonically correct for the addition of viable cells. CyGEL™ will now transit from sol to gel at 20-21°C, usually in 1-2 minutes.

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

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

1. Cool an unused vial of PBS-primed CyGEL™ on ice.
2. Prepare cells for mounting in CyGEL™: 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™.
4. Transfer 250ul of the CyGEL™/cell suspension into a cold P1000 pipette tip. Quickly dispense onto a clean microscope slide by streaking along the surface for a length similar to the coverslip to be applied (see fig. 1). Repeat the process for the remaining CyGEL™/cell suspension – giving two microscope slide preparations.
5. Apply a coverslip to each CyGEL™/cell suspension (see fig. 2).
6. Place the microscope slide onto an ice pack to allow the CyGEL™ to liquefy. The sample will then spread out under the coverslip. Remove the slide from the ice pack. The CyGEL™ will now re-gel as it reaches room temperature. The sample is now ready for visualization.

CyGEL™ 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
- Excipients modify the viscosity and the sol:gel transition temperature
- Recommended max. dilution: 20% v/v

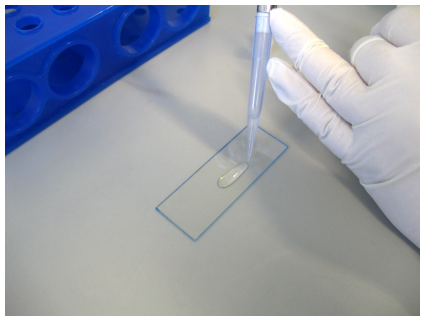

Fig. 1 Using a P1000 pipette tip streak cold CyGEL™ along the slide for the length of a coverslip

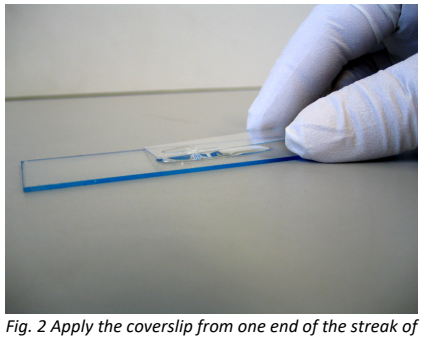

Fig. 2 Apply the coverslip from one end of the streak of CyGEL™ taking care not to trap bubbles. One applied, cool the slide on an ice-pack to allow the CyGEL™ to liquefy and spread under the coverslip.

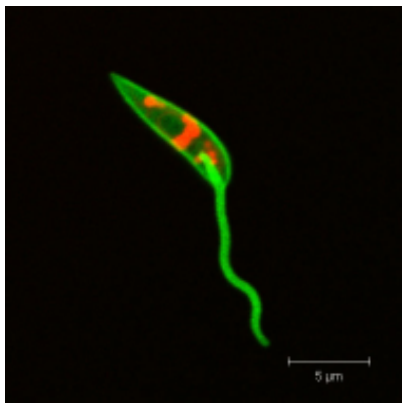

Fig.3 Live Leishmania major immobilized and imaged in CyGEL™. Image courtesy Dr H Price, Univ of York

PROTOCOL 3:

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

1. Cool an unopened 500 ul tube of PBS-primed CyGEL™.
2. Pipette 2.05 µl DRAQ5™ (5 mM stock) and dispense into the CyGEL™ 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™ 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 PBS-primed CyGEL™.
2. Pipette 5.1 µl DRAQ7™ stock solution and dispense into the PBS-primed CyGEL™ 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.

   This can also be achieved with propidium iodide. Prepare a 1mg/ml solution. Add 2.56 µl to an unused vial of primed CyGEL.

PROTOCOL 5:

CyGEL™ MOUNTING OF CELLS IN A CHAMBER COVERSLIP FOR MICROSCOPY

1. Cool an unopened vial of PBS-primed CyGEL™ 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 PBS-primed CyGEL™ 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™ layer will set thereby immobilizing cells and cell clusters for visualization.

It is possible to create wells for CyGEL™ using microscope slides and silicone o-rings (i.d. 8-10 mm). Smear Silicone (vacuum) grease onto the o-ring and bed down onto the slide. This creates a well that can be filled with CyGEL™. If bedded onto a coverslip-bottomed petri-dish, the o-ring can be 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
Price, H.P., et al. (2010) Molec. Biochem. Parasitol. 169: 66-69
Robertson, F.M., et al. (2010) J. Biomolec. Screen. 15: 820-829
Edward, R., (2010) Am. Biotech. Lab. July/Aug: 12-14

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