

CyGEL™ - EXAMPLE PROTOCOLS

A novel thermoreversible hydrogel mountant for LIVE cells

THE FOLLOWING PROTOCOLS ARE AVAILABLE:

- PREPARATION OF CyGEL™ FOR USE
- CyGEL™ MOUNTING OF CELLS ONTO A STANDARD MICROSCOPE SLIDE FOR CONFOCAL MICROSCOPY / CELL IMAGING
- CyGEL™ AS A DELIVERY MEDIUM FOR A CELL-PERMEANT DYE (DRAQ5™) IN FLUORESCENT IMAGING OF ADHERENT CELLS
- CyGEL™ AS A DELIVERY MEDIUM FOR A CELL-IMPERMEANT DYE (e.g. PROPIDIUM IODIDE) IN TIME-LAPSED FLUORESCENT IMAGING OF MEMBRANE-COMPROMISED CELLS
- CyGEL™ MOUNTING OF CELLS IN A CHAMBER COVERSIP FOR CONFOCAL MICROSCOPY / CELL IMAGING

- and are provided in this document.

General tips for use of CyGEL™:

Read the material safety data sheet supplied prior to use of CyGEL™

CyGEL™ changes from a sol (chilled) to a gel (warmed) quickly, aiding rapid sample preparation and immobilisation of cells for visualisation.

CyGEL™ becomes a gel at a convenient temperature (nominally room temperature).

CyGEL™ is formulated to maintain cellular integrity for periods of ≥ 1 h for most cell types and often much longer.

Keep CyGEL™ stocks on ice to facilitate convenient pipetting.

If required, ice cold water can be used to "clear" clogged / blocked tips.

P1000 micropipette displacement tips are recommended for handling CyGEL™.

CyGEL™ can accept additives such as fluorescent DNA dyes (e.g. DRAQ5™, propidium iodide). These should be added when CyGEL™ is chilled (sol). Additions of 1:100 - 1:20 should not affect the performance of CyGEL™ but each new additive should be tested for impact on gel formation / stability. (Examples can be found at www.biostatus.com).

The overall cell/bead/dye suspension volume should not dilute CyGEL™ by more than 10%. Otherwise, the integrity of the hydrogel will be compromised.

CyGEL™ is intended for research purposes only.

PROTOCOL 1: PREPARATION OF CyGEL™ FOR USE

Reagents required:

- CyGEL™
- 40X PBS (supplied)
- microcentrifuge tubes
- Pipette tips
- ice pack/bath

Read the supplied [Material Safety Data Sheet](#) prior to handling CyGEL™.

As supplied, CyGEL™ will transit from sol to gel at approximately 23°C

1. Select a vial of CyGEL™.
2. Cool the vial on ice for 1-2 minutes.
3. 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 at the correct isotonic strength for the addition of viable cells.

The PBS-primed CyGEL™ will now transit from sol to gel at approximately 20-21°C.

PROTOCOL 2: CyGEL™ MOUNTING OF CELLS ONTO A STANDARD MICROSCOPE SLIDE FOR CONFOCAL MICROSCOPY / CELL IMAGING

Reagents required:

- PBS-primed CyGEL™ (from Protocol 1)
- chilled water
- chilled pipette tips
- microcentrifuge tubes
- ice pack
- microscope slides
- coverslips
- cell buffer e.g. PBS

1. Cool the 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 (typically 1 - 5 x 10⁵ cells depending upon the application).
3. Pipette the cell suspension into the vial containing PBS-primed CyGEL™.
4. Transfer 250µl 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 approaching that of the coverslip to be applied. Repeat the process for the remainder of the CyGEL™/cell suspension – giving two microscope slide preparations.
5. Apply a coverslip to the CyGEL™/cell suspension.
6. Place the microscope slide onto an ice pack to allow the CyGEL™ to liquefy. The sample will then spread out under the coverslip.

7. Remove the slide from the ice pack. The CyGEL™ will now re-set as it reaches room temperature. The sample is now ready for visualisation.

NOTE: The total volume of cells, beads and dyes added to the PBS-primed CyGEL™ should never exceed 10% v/v

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

Reagents required:

- PBS-primed CyGEL™ (from Protocol 1)
 - chilled water
 - chilled pipette tips
 - Microcentrifuge tubes
 - cell / nuclear dye e.g. DRAQ5™
1. Read the supplied [Material Safety Data Sheet](#) prior to handling CyGEL™ and DRAQ5™.
 2. Cool the tube of PBS-primed CyGEL™.
 3. 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.
 4. 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. PROPIDIUM IODIDE) IN TIME-LAPSED FLUORESCENT IMAGING OF MEMBRANE-COMPROMISED CELLS

Reagents required:

- PBS-primed CyGEL™
 - chilled water
 - chilled pipette tips
 - Microcentrifuge tubes
 - cell impermeant dye e.g. propidium iodide (PI), PBS
1. Read the supplied [Material Safety Data Sheet](#) prior to handling CyGEL™ and DRAQ5™.
 2. Cool the tube of PBS-primed CyGEL™.
 3. Prepare a 1mg/ml stock solution of propidium iodide.
 4. Pipette 2.56 µl propidium iodide stock sol. and dispense into the PBS-primed CyGEL™ and mix thoroughly. Propidium iodide is now at a concentration of 5 µg/ml, sufficient for stoichiometric chromatin binding.
 5. 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 PI and will appear fluorescent under excitation with the appropriate wavelength.

PROTOCOL 5: CyGEL™ MOUNTING OF CELLS IN A CHAMBER COVERSILIP FOR CONFOCAL MICROSCOPY / CELL IMAGING

Reagents required:

- PBS-primed CyGEL™
 - chilled water
 - chilled pipette tips
 - ice pack
 - microscope 8-chamber coverslip (e.g. Nunc)
 - cell buffer e.g. PBS
1. Read the supplied [Material Safety Data Sheet](#) prior to handling CyGEL™.
 2. Cool a vial of PBS-primed CyGEL™ on ice / ice pack.
 3. Prepare cell suspension: Wash the cells in buffer (e.g. PBS) by centrifugation. Resuspend the cell pellet in the same buffer at a suggested concentration of 2×10^6 cells/ml.
 4. Pipette 25 μ l of the cell suspension into a clean chamber of a microscope 8-chamber coverslip.
 5. Transfer 250 μ l 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 visualisation.

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