CyGEL™ Sustain - EXAMPLE PROTOCOLS

A novel thermoreversible hydrogel mountant for extended imaging of LIVE cells

bio status LIMITED

THE FOLLOWING PROTOCOLS ARE AVAILABLE:

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

- and are provided in this document.

General tips for use of **CyGEL™ Sustain**:

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

CyGEL™ Sustain changes from a sol to a gel at 23-24°C, aiding rapid sample preparation and immobilisation of live cells for visualisation.

CyGEL™ Sustain is formulated to accept concentrated culture medium (e.g. RPMI) to maintain cellular integrity for periods of ≥ 2 h for most cell types and often much longer.

Keep **CyGEL™ Sustain** stocks refrigerated 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™ Sustain.**

CyGEL™ Sustain can accept additives such as fluorescent DNA dyes (e.g. DRAQ5™, propidium iodide). These should be added when **CyGEL™ Sustain** is chilled (sol). Additions of 1:100 - 1:20 should not affect the performance of **CyGEL™ Sustain** 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™ Sustain** by more than 5%. Otherwise, the integrity of the hydrogel will be compromised.

CyGEL™ Sustain is intended for research purposes only.

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PROTOCOL 1: PREPARATION OF CyGEL™ Sustain FOR USE

Reagents required:

- **CyGEL™ Sustain**
- 10X RPMI (See note a. below for formulation)
- Microcentrifuge tubes
- Pipette tips
- ice pack/bath

Read the supplied Material Safety Data Sheet prior to handling **CyGEL™ Sustain.**

As supplied, **CyGEL™ Sustain** will transit from sol to gel at approximately 23-24°C

1. Remove a fresh vial of **CyGEL™ Sustain.**
2. Chill the vial on ice.
3. Add 87.5 µl RPMI-stock (as prepared in note a. below) to the **CyGEL™ Sustain.** Mix thoroughly, taking care to avoid bubble formation.

The RPMI-primed **CyGEL™ Sustain** is now at the correct isotonic strength for the addition of viable cells, bead and dyes up to a maximum volume of 5% v/v.

The RPMI-primed **CyGEL™ Sustain** will now transit from sol to gel at approximately 23-24°C.

Note a.
Preparation of RPMI culture medium for dedicated use with **CyGEL™ Sustain**

To 20 ml of 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

PROTOCOL 2: CyGEL™ Sustain MOUNTING OF CELLS IN A CHAMBER COVERSLIP FOR CONFOCAL MICROSCOPY / CELL IMAGING

Reagents required:

- RPMI-primed **CyGEL™ Sustain** (from Protocol 1)
- microcentrifuge tubes
- ice pack
- microscope 8-chamber coverslip (e.g. Nunc)
- cell buffer e.g. RPMI

1. Read the supplied Material Safety Data Sheet prior to handling **CyGEL™ Sustain.**
2. Cool a vial of RPMI-primed **CyGEL™ Sustain** to below room temperature.

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3. Prepare cell suspension: Wash the cells in buffer (e.g. RPMI) by centrifugation. Resuspend the cell pellet in the same buffer at a suggested concentration of 4×10^6 cells/ml.
4. Pipette 12 µl of the cell suspension into a clean chamber of a microscope 8-chamber coverslip.
5. Transfer 250 µl of the RPMI-primed **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 visualisation.

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

Reagents required:

- RPMI-primed **CyGEL™ Sustain** (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™ Sustain** and DRAQ5™.
2. Cool a vial of RPMI-primed **CyGEL™ Sustain** below room temperature.
3. Add 2.35 µl DRAQ5™ (5 mM stock) 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 binding should completely equilibrate after 60-80 min. However, imaging of nuclei will be possible 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. PROPIDIUM IODIDE) IN TIME-LAPSED FLUORESCENT IMAGING OF MEMBRANE-COMPROMISED CELLS

Reagents required:

- RPMI-primed **CyGEL™ Sustain**
- chilled water
- chilled pipette tips
- Microcentrifuge tubes
- cell impermeant dye e.g. propidium iodide (PI), PBS

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1. Read the supplied Material Safety Data Sheet prior to handling **CyGEL™ Sustain** and DRAQ5™.
2. Cool the tube of RPMI-primed **CyGEL™ Sustain**.
3. Prepare a 1mg/ml stock solution of propidium iodide.
4. Add 2.94 µl propidium iodide stock solution to the RPMI-primed **CyGEL™ Sustain** 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.

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