Cell-Mate3D™ Whole Mount Staining and Imaging Protocol

Use this protocol as a guide to stain and visualize cells in the Cell-Mate3D™ matrix. This is an example protocol that employs phalloidin, 53BP1 antibody, and DAPI. Concentrations of antibodies and stains may require optimization.

REQUIRED EQUIPMENT

Water bath
1.5mL microfuge tubes
Blunt end forceps
1mm thick cover slip
Inverted confocal microscope

REQUIRED REAGENTS

- 1X PBS, pH7.4
- Phalloidin – Alexa Fluor 488 (Biolegend #103015)
- Primary antibody: Mouse anti human 53BP1 (nuclear) (EMD Millipore #MAB3802)
- Secondary antibody: Goat anti mouse - Alexa Fluor 568 (Thermo Fisher A-21124)
- NucBlue fixed cell stain ready probes DAPI (Life Technologies #R37606)
- Fixation solution: 4% Paraformaldehyde
- Permeabilization solution: PBS-Triton X 100 (0.5%)
- Blocking solution: PBS-BSA (1%)
- Wash buffer: PBS-Tween 20 (0.1%)
- Antibody dilution buffer: PBS-Tween 20 (0.1%) + BSA (0.5%)

Note: All incubations and washes must be carried out on a shaker to allow movement in the media. We recommend gentler shaking for antibody incubations and slightly more vigorous shaking for the washes.

1) Fix a $0.05cm^3$- $0.1cm^3$ sized Cell-Mate3D™ sample in 4% Paraformaldehyde for 3.5 hours

1) After fixing, the cocoon will become very brittle. Gently break up the Cell-Mate3D™ sample into $2mm^3 - 3mm^3$ pieces and wash 2 x 5 minutes in PBS in a 1.5mL microfuge tube
2) Divide them evenly into 4-5 portions. They can be stored in 1.5mL microfuge tubes with 1mL PBS for over a week.

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To stain a matrix piece:

3) Permeabilize sample with 500uL PBS-Triton X (0.5%) for 10 minutes
4) Wash 2 x 5 minutes in 1mL PBS and aspirate all liquid
5) Block sample in 1mL PBS-BSA (1%) for 1 hour or overnight (depending on the antibody)
6) Dilute primary antibody (1:400) in PBS-Tween (0.1%) + BSA (0.5%) and incubate in a final volume of 400uL for 1 hour
7) Wash sample 3 x 5 minutes in 1mL PBS-Tween (0.1%)
8) Dilute secondary antibody (1:500) and Phalloidin (1:200) in PBS-Tween (0.1%) + BSA (0.5%) and incubate for 45 minutes in a final volume of 400µL
9) Wash 3 x 5 minutes in 1mL PBS-Tween (0.1%)
10) For DAPI staining, replenish the tube with 500µL of PBS and add two drops of DAPI. Incubate for 5 minutes
11) Replace the liquid with fresh PBS. Cells are best imaged directly after staining. Keep away from light.
12) Images are best taken with an inverted confocal microscope. Place the sample on a 1mm thick cover slip and keep moist with PBS. Gently place a cover slip on top of the sample and image.

SAFETY DISCLAIMER:
Only competent and trained personnel using appropriate personal protective equipment and working within a controlled environment should handle all chemicals and perform the protocol described herein. Prior to performing this protocol, users should review appropriate safety information, including the manufacturers MSDS, related to the components used in this protocol. Bioactive Regenerative Therapeutics, Inc. shall not be held liable for any loss, injury or damage as a result from the use of this protocol.

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