Cell-Mate3D™ Cryosectioning and Staining

Use this protocol to cryosection and stain cells in Cell-Mate3D™.

REQUIRED EQUIPMENT

- Eppendorf Tubes
- Forceps
- Coplin Jar
- Cryo Mold
- OCT Mounting Medium
- Razor Blades
- Pencil
- Paintbrushes
- Superfrost Plus Slides, Positively Charged (Thermo Fisher #6776214)
- Cryostat
- Inverted Confocal Microscope

REQUIRED REAGENTS

- PBS
- DI Water
- Liquid Nitrogen/Dry Ice
- Conjugated or Unconjugated Antibodies
- Isopentane (Sigma #270342)
- Blocking Buffer: 100% Normal Human Serum – Unconjugated (Jackson ImmunoResearch Laboratories #009-000-121), Tween 20 (Sigma #9416),
- NucBlue Fixed Cell Stain Ready Probes DAPI (Thermo Fisher #R37606)
- Vectashield Antifade Mounting Medium (Vector Laboratories #H-1000)

PROTOCOL

1. Sample Preparation
   a) Remove sample from culture media and briefly wash in PBS.
   b) Dry the sample lightly with a paper towel or Kimwipe.
   c) Place Cell-Mate3D™ sample in mold with OCT in preferred orientation.
   d) Cover the Cell-Mate3D™ sample with OCT, avoiding air bubbles in the mounting medium.
   e) Both flash freezing and slow freezing of the Cell-Mate3D™ sample has shown positive results. Samples can be flash frozen with OCT in an isopentane slurry, slow frozen with OCT overnight at -80˚C, or by using an alternative freezing method.

www.BRTILifeSciences.com info@BRTILifeSciences.com (855) 849-BRTI (2784)

Rev C 5/2016

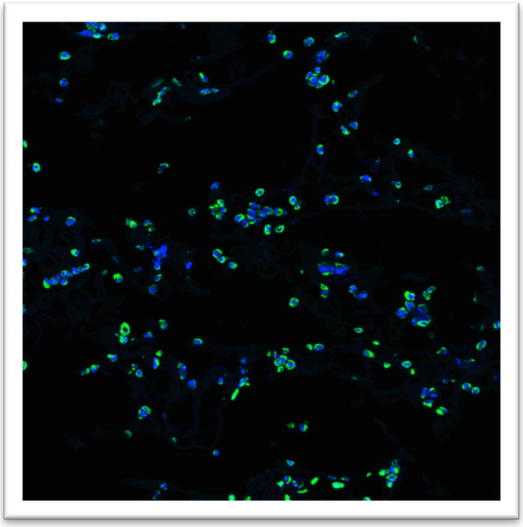
2. Staining & Storing
   a) Thaw slides at room temperature for 15 min.
   b) Rehydrate slides in PBS for 10 minutes using a Coplin jar.
   c) Gently wash the slides in DI water for 1 minute.
   d) Block slides for 30 minutes at room temperature (with 100uL of 10% HSA, PBS, 0.3% Tween 20 or 100% HSA). Cover the top of each slide with parafilm to reduce evaporation.
   e) Stain the slides for 1 hour at room temperature, shielded from the light (inverted tip box with damp napkin on bottom. Use 100 µl of primary-antibody solution for each coverslip (blocking solution + antibody). Cover the top of each slide with parafilm to reduce evaporation.
   f) Gently wash the slides 3 times in PBS for 5 minutes.
   g) Remove any drops of PBS on the slide with a Kimwipe before mounting as it may dilute out the mounting medium and lower its refractive index.
   h) Mount with Vectashield mounting medium or just add DAPI to the slides. For best results, image immediately using a Confocal Microscope.
   i) Seal the edges of the coverslip with nail polish and allow to air dry.
   j) Store in 4C until ready to image.

Cryosection & Staining Tips:

- Avoid bubbles in the OCT especially near the sample as it will cause the sample to fold on itself.
- If sample requires fixation step, 10% Neutral Buffered Formalin has given better results than 4% PFA.

www.BRTILifeSciences.com info@BRTILifeSciences.com (855) 849-BRTI (2784)

Rev C 5/2016



HeLa cells embedded in Cell-Mate3D™ cryosectioned and stained with CD44-A488 (VWR# 103016-BL) and DAPI. Sample was flash frozen in an isopentane slurry. 100% HSA used for blocking reagent. Imaged with inverted confocal microscope. 20X magnification.

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.