

Cell-Mate3D™ Viability Staining Protocol

Use this protocol to visualize viability (live and dead) cells in Cell-Mate3D™ matrix.

REQUIRED EQUIPMENT

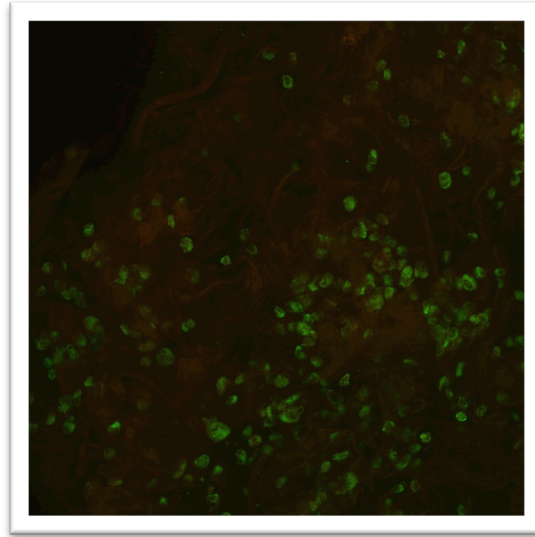
- 24-well plate
- 22 x 50mm Cover Glass (#1.5)
- Inverted Confocal Microscope
- Forceps and scissors/blade

REQUIRED REAGENTS

- Cell Trace Calcein Green, AM (Thermo Fisher #C34852)
- Ethidium Homodimer-1 (Thermo Fisher #E1169)
- Sterile water
- PBS
- DMSO
- Cell-Mate3D™ cultures

PROTOCOL

1. Wash Cell-Mate3D™ matrix in 1mL of PBS, briefly and cut into thin sections (~1-2 mM thick).
2. Prepare the following staining solutions as follows:
 - a. Reconstitute Ethidium Homodimer-1 (EthD-1) with 80% DMSO in water for a stock concentration of 1mM. Aliquot and store in -20°C.
 - b. Reconstitute 1 vial of Calcein Green AM (50ug) with 12.7µL of DMSO for a stock concentration of 5mM. This stock will be stable at -20°C for up to 6 months.
 - c. In a 24-well plate, combine 1µL of 5mM Calcein Green, AM (1:2000, 2.5µM) concentration) with 4µL of 1mM EthD-1 (1:333, 3µM) in 1mL of PBS.
3. Transfer 1-2 sections of the Cell-Mate3D™ matrix into the prepared well of a 24-well plate and incubate for 10 minutes at 37°C, 5% CO₂. Incubation time and working concentrations may require optimization depending on cell type.
4. Wash the stained sections of Cell-Mate3D™ matrix in 1mL of PBS for 1 minute.
5. Place sample on a cover slip with a drop of PBS. Cover the sample with a cover slip to prevent the sample from drying out.
6. Visualize samples through FITC and TRITC channels.



Live/Dead Staining. HeLa Cells were embedded into a 500 μ L Cell-Mate3D™ matrix. A section of this construct was stained and imaged using the method described herein.

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.