

Matrix Immobilization: Plate Bottom Protocol

Use this protocol to generate a layer of cell-embedded Cell-Mate3D™ cultures on the bottom of a 48-well plate.

*Note: The Cell-Mate3D™ culture setup varies from the technical data sheet to enable the matrix to spread over the bottom of the well. Importantly:

- A standard dry blend is hydrated with 300µL of hydration fluid
- A large dry blend is hydrated with 600µL of hydration fluid

EQUIPMENT REQUIRED

- Centrifuge & Plate Adapters
- Cell Culture/Laminar Flow Hood
- P1000 pipettor and tips

REAGENTS REQUIRED

- Non-treated 48-well cell culture plate (e.g. Eppendorf #0030723015)
- 1mL syringe (e.g. BD Biosciences #309628)
- Cell-Mate3D™ Kit (BRTI Life Sciences #CM-1001 or #CM-1002)

PROTOCOL

- 1) Using the technique described in the Cell-Mate3D™ Technical Data Sheet, hydrate the Cell-Mate3D™ dry blend with 300µL (Standard Kit) or 600µL (Large Kit) of hydration fluid containing the desired cell population. Note: The volume of hydration fluid used in this protocol deviates from the amount used in the Technical Data Sheet.
- 2) Cut 50µL size Cell-Mate3D™ matrix pieces into each well.
- 3) Remove plunger from the 1mL syringe. Gently spread the gel evenly on the bottom of the plate with the plunger.
- 4) Spin the plate at 1,280g with the brakes OFF for 1.5 minutes.
- 5) Gently add appropriate cell culture media to each well.
- 6) After 24hrs, the cell-embedded Cell-Mate3D™ gel layer will float. Remove all media except 100µL and repeat step #4.
- 7) Gently replenish with fresh media.
- 8) The gel layer can be easily removed with tweezers and processed for further analysis.



Figure 1. Cell-Mate3D™ culture on a plate bottom. A 50µL piece of Cell-Mate3D™ embedded with 2 Million HeLa cells was spread and centrifuged in the bottom of a 48-well plate.

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