BRTI™ Life Sciences

Cell-Mate 3D™

Technical Data Sheet

Standard Kit
CM-1001

Large Kit
CM-1002

Cell-Mate3D™ Technical Data Sheet

Materials supplied:

- Dry Blend—Store at -20°C
- Hydration Fluid—Store at 4°C
- Formatting tool with short plunger
- Long plunger

Materials not supplied:

- Vortex (set at maximum setting)
- Sterile spatula
- Sterile blunt end tweezers
- Sterile dissection scissors or scalpel
- Optional: 1cc BD syringe #309628
- Optional: Needle 19-23G

Before beginning:

- Bring Dry Blend and Hydration Fluid to room temperature
- Place Vortex in laminar flow hood
- Set Vortex to maximum agitation
- **View Cell-Mate3D™ video at www.BRTILifeSciences.com/about-cell-mate/**

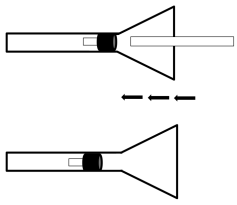
Embedding Cells into Cell-Mate3D™: Creation of Cell-Mate3D™ Matrix

Working quickly under sterile conditions (cell culture/laminar flow hood):

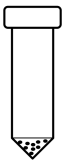
1. **Prepare dry blend:** Centrifuge dry blend by ramping up to 280 x G. Once centrifuge reaches 280 x G stop the centrifuge using high break.
2. **Prepare formatting tool:** Remove formatting tool and long plunger from tube. Gently push the short plunger in the formatting tool down about 2mm with the long plunger to "unstick" the short plunger and place the formatting tool back in the tube. (Fig1)
3. Harvest and pellet cells according to established procedures for cell type.
4. Resuspend cells in hydration fluid to reach a total volume of 250µL (Standard Kit) or 500µL (Large Kit) (Fig 2).
5. Vortex dry-blend under maximum agitation for at least 10 seconds.
6. Take 250µL (Standard Kit) or 500µL (Large Kit) of cell + Hydration Fluid solution, and ***pipet into the dry blend while vortexing the dry blend under maximum agitation*** (Fig 3). This action forms the Cell-Mate3D™ matrix (Fig 4).
7. Using a sterile spatula, remove the cell-embedded matrix and place in the top of the formatting tool (Fig 5).
8. Brief centrifugation: Ramp up centrifuge to 1,520 x G (about 45 sec total) and **immediately** stop centrifuge using high brake (Fig 6). This action forms the Cell-Mate3D™ matrix within the formatting tool (Fig 7).
9. Remove the funnel from the stem of the formatting tool. Using the long plunger, extrude the formed matrix out of the stem by pushing on the short plunger (Fig 8, Fig 9) and thus pushing out the matrix.
10. The matrix may be extruded directly into a 1cc syringe for injection or as the matrix is being extruded, it may be cut into 3-5 equal parts using sterile scissors or a scalpel.
11. Culture the cell-embedded gel as desired. The matrix will float, and must be fully submerged in the culture dish.

Cell-Mate3D Overview:

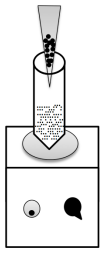
Prepare formatting tool: Remove formatting tool and long plunger from tube. Gently push the short plunger in the formatting tool down about 2mm with the long plunger to "unstick" the short plunger and place the formatting tool back in the tube.



Harvest cells, pellet, and resuspend in Hydration Fluid



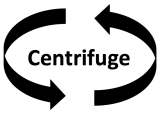
Pipet Cell+Hydration Fluid Solution into the dry blend ***while vortexing the dry blend under maximum agitation***



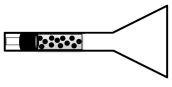
Cell-Mate3D™ matrix is formed

Remove the cell-embedded matrix using a sterile spatula and place in the top of the formatting tool

Ramp up centrifuge to 1520 x G (about 40 sec total) and **immediately** stop centrifuge using high brake



Cell-Mate3D™ matrix is formed in the formatting tool



Remove the funnel from the formatting tool. Using the long plunger, extrude the formed matrix by pushing on the short plunger and thus pushing out the matrix. Culture as desired.

General Considerations

- The minimum and maximum number of cells that will be compatible with Cell-Mate3D™ should be optimized to each cell type. Guidelines here are for human Mesenchymal Stem Cells, which are approximately 20 microns in length. In this case, use 9.5M cells for a 250µl matrix and 19M cells for a 500µL matrix.

- If too many cells are loaded into the material, the material may not solidify properly, making culture more difficult. If too few cells are loaded into the material, they may be difficult to find when performing microscopy, or may be too sparse to interact with each other.

- Vigorously mixing the dry blend using a vortex set at the maximum setting 10 seconds before *and* during the addition of cells+Hydration Fluid is important for proper formation of the Cell-Mate3D™ matrix.

Troubleshooting Guide

Problem	**Action**
The cell-embedded gel does not go down into the funnel stem during brief centrifugation.	• "Unstick" the short plunger by gently moving it down 2mm with the long plunger. • Increase centrifuge speed by 200 RPM.
Cell-embedded gel is less than 400µl after centrifugation.	• Be sure that all of the dry-blend is at the bottom of the tube before beginning • Be sure to vigorously vortex dry blend before and during addition of cells+Hydration Fluid. The total mass of dry blend should be in-motion during the addition of cells+Hydration Fluid

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Frequently Asked Questions

What is Cell-Mate3D™ and How Does it Form?
Cell-Mate3D™ matrix is comprised of 2 main components: Hyaluronic acid (HA) and Chitosan (CT). HA is a linear polysaccharide found in the extracellular matrix (ECM) during embryonic development and wound healing. CT, derived from Chitin (deacetylated amines), is a positively charged polysaccharide that is found in the exoskeleton of crustaceans. Both HA and CT are natural occurring, biocompatible, polymers that have been used separately in many biomedical applications. When hydrated with Hydration Fluid containing cells, PEC fibers form through electrostatic interactions of HA^{COO-} groups and $CT^{NH_3^+}$ groups, giving the matrix its structure. Cells embedded in Cell-Mate3D™ matrix can be cultured *in vitro* or injected into animal models for *in vivo* applications.

Do I need a cross-linking reagent or exposure to UV light to form the matrix?
No! Cell-Mate3D™ is designed to be free of cross-linking reagents and exposure to UV light, which can be harmful to cells and increase set up time.

Are my cells attached to the Cell-Mate3D™ matrix, or each other?
Both! The Cell-Mate3D™ matrix is designed to promote both cell-cell and cell-matrix interaction within a 3D environment.

How many cells will embed into a Cell-Mate3D™ matrix?
This will need to be determined for each cell type depending on the size of the cells, and if cells are single cells or aggregates. For hMSCs as single cells, and iPSCs as colonies, about 19M cells will embed into a 500µl matrix and about 9.5 million cells will embed into a 250µl matrix.

Do I need to use a special media with Cell-Mate3D™?
No. Please culture your Cell-Mate3D™ matrix in media that best suits your application.

Will the Cell-Mate3D™ matrix break down over time?
While the Cell-Mate3D™ matrix should not dissociate in culture, the material itself may be broken down and metabolized by cells over time, leaving less Cell-Mate3D™ material and more cells, cell secreted ECM, and 3D cell structures.

What is the porosity of the Cell-Mate3D™ matrix?
Because Cell-Mate3D™ is a hydrocolloid, consisting of an insoluble "dispersed" phase and a soluble "dispersion" phase, the concept of porosity does not apply to this material. That is, every portion of the hydrated matrix has mass. However, if too many cells are added to the material, the HY and CT molecules will not form strong bonds, causing the material to have weak mechanical

properties. If too few cells are added, then identifying the cells through microscopy may prove difficult. Therefore, the appropriate number of cells per matrix volume will need to be determined for each cell type.

Do I need to use the Hydration Fluid provided in the kit?
Yes. Use of a different hydration fluid will dramatically decrease the volume of your Cell-Mate3D™ matrix, and thus the ability of your cells to integrate into the matrix.

Can I re-use my formatting tool?
Yes. Cell-Mate3D™ formatting tools can be autoclaved. Be sure to clean well after use before autoclaving.

I am seeing autofluorescence and background staining with my Cell-Mate3D™ sample. Is there a way to minimize this?
We recommend using inverted confocal microscopy to image samples. See optimized staining and imaging protocols at www.BRTILifeSciences.com

Which microscopy techniques work best with Cell-Mate3D™?
Inverted confocal microscopy works well. See optimized staining protocols at www.BRTILifeSciences.com

I'm doing cancer research. How many cells should I embed into Cell-Mate3D™? It is best to optimize cell number for your specific cell type. We recommend 9.5M cells per 250µL matrix as a starting point and observing for desired outcome over 7-21 days. Adjust cell population as needed.

I have cells that require a specific ECM component. Can I add additional ECM from an existing protocol into Cell-Mate3D™?
Yes, you can replace up to 20% of the hydration fluid with your ECM component. However, mechanical properties of the construct may change, leading to a softer product.

Do I have to use the formatting tool or can I flatten the Cell-Mate3D™ matrix on the bottom of a cell culture plate?
Our recommendation is to use the formatting tool, extrude out a desired matrix piece, (30-100µL) and cut so that the matrix piece is transferred into the desired culture plate containing media.

Can I use a DAPI stain with Cell-Mate3D™?
Yes, DAPI will stain nuclei embedded in Cell-Mate3D™.