Islet-Mate™

8 Well micro gel Size: ~35µL
IM-1

48 Well Plate Sizes: ~70µL and ~140µL
IM-3 and IM-6

Technical Data Sheet

Islet Mate™ *Beta Test Kit* for **(IM-1, IM-3, and IM-6 Kits)**

Rev H - May 28, 2019

Technical Data Sheet— Chitosan Hydrogel (IM-1, IM-3, and IM-6)

Materials supplied:

- Dry Construct - Store at 4°C
- Hydration Fluid - Store at 4°C
- Diluant Fluid (sterile water)
- Optional: Long-stem funnel with short plunger
- Optional: Long plunger

Materials not supplied:

- Sterile spatula
- Sterile blunt end tweezers
- Sterile dissection scissors
- Optional: 1cc BD syringe #309628
- Optional: Needle 18-20G

Before beginning:

- Bring plate or micro wells of dry constructs and hydration fluid to room temperature

Embedding Cells into Islet Mate:

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

Islet Mate constructs come in three sizes:

IM-1 is a ~35uL prepared in a 96 well plate (micro well)
IM-3 is a ~70uL prepared in a 48 well plate
IM-6 is a ~140uL prepared in a 48 well plate

1. Prepare Plate: (8 well strip or 48 well plate)
   1.1. Warm hydration fluid and cell culture media in a 37°C water bath for at least 30 minutes.
   1.2. Take Islet Mate™ kit out of fridge (4°C) and warm to room temperature at least 30 minutes.
   1.3. Under sterile conditions. Remove the 8 well row or the 48 well plate from package.
   1.4. Remove cap/cover plate from the microwell(s)/plate intended to hydrate with cells.

2. Prepare Cells:
   2.1. Harvest and pellet cells according to established procedures for cell type.
   2.2 Re-suspend cells (IEQ) in hydration fluid and diluent (as needed) to reach a cell suspension (cells + hydration fluid) solution volume 35µL (for IM-1), 70µL (for IM-3), and 140µL (for IM-6).

3. Embed Cells:
   3.1. **Use the table below** by pipetting the required amount of Hydration Fluid with Diluent (if necessary) and cell suspension directly on top of channel opening of dry construct.
   3.2. Allow construct to cure for up to 5 minutes prior to transferring to culture plate

IM – 1 ~35µL Table for Hydration Fluid for			
Gel Stiffness	Fluid Hydration µL	Diluent µL	Total Volume µL
Stiff	23	5	35
Less Stiff	11	15	35
Soft (Injectable)	8	20	35

Cells + cell suspension volume assumed to be approximately 10µL

IM – 3 ~70µL Table for Hydration Fluid for			
Gel Stiffness	Fluid Hydration µL	Diluent µL	Total Volume µL
Stiff	69	0	70
Less Stiff	33	20	70
Soft (Injectable)	24	30	70

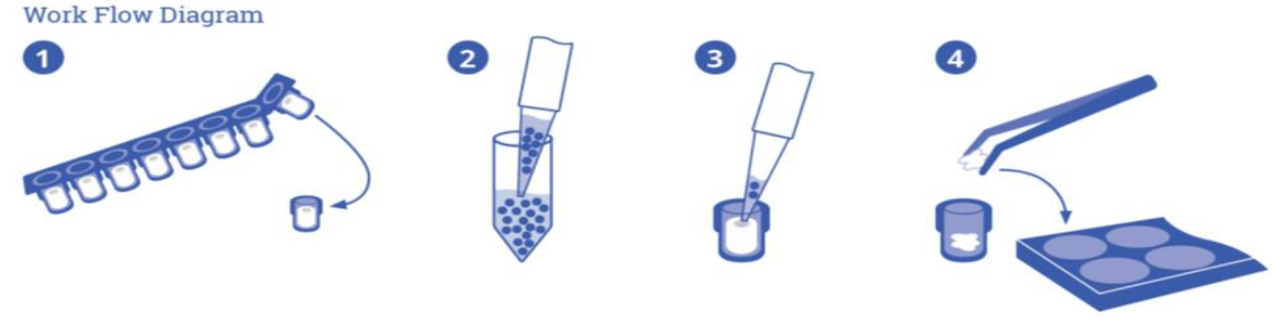
Cells + cell suspension volume assumed to be approximately 25µL

IM – 6 ~140µL Table for Hydration Fluid for			
Gel Stiffness	Fluid Hydration µL	Diluent µL	Total Volume µL
Stiff	120	0	140
Less Stiff	95	40	140
Soft (Injectable)	69	60	140

Cells + cell suspension volume assumed to be approximately 50µL

4. Transfer Gel:
   4.1 With a sterile spatula/tweezers gently transfer newly created gel from the microwell to desired well plate (6/12/24/48). **Injection application:** Reference page 4 for preparation.
   4.2 Incubate plate in 37°C/5% CO2/95% humidity to maintain cells or proceed with desired downstream experiments.

WORK FLOW FOR 8 WELL (micro gel kits) for the 48 WELL PLATE *



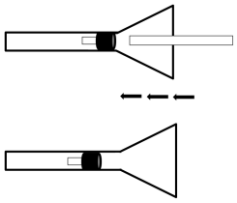
* For work flow in 48 well place replace images #1 and #3 with 48 well plate.
Hydration of construct within the well.

SPECIAL APPLICATION:

Preparation and Setup for Injection Application using Islet Mate™

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

1.

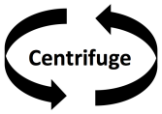


Remove the cell-embedded matrix using a sterile spatula and place in the top of the long-stem funnel

2.

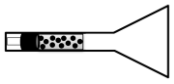
Ramp up centrifuge to 1,000 g (or 2,800 RPM) (about 40 sec total) and **immediately** stop centrifuge using high brake

3.



Matrix is formed in the funnel stem

4.

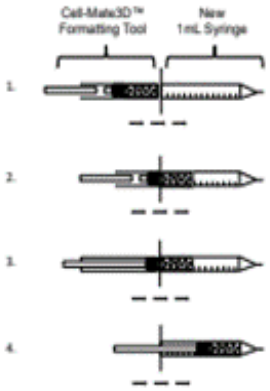


Remove the funnel. Using the long plunger, extrude the formed matrix out of the funnel stem by pushing on the short plunger and thus pushing out the matrix. Culture as desired.

5.

6.

Transfer the formed gel into a 1cc syringe with needle. Now your prepared for injection.



Troubleshooting

1. If the channel to the hydrogel does NOT fully close. Use sterile spatula or forceps to gently close the opening by pushing on one side of the hydrogel to collapse or close the channel. The hydrogel is tacky and will stick together.
2. Do not place un-hydrated plates in the incubator to warm as condensation will initiate the construct hydration process.
3. The minimum and maximum number of cells compatible with Islet Mate are dependent upon the construct size. Follow the hydration instruction on the package.
4. Introducing the cell suspension solution directly over the channel ensures that the most number of cells will be loaded into the construct.
5. If desire softer Islet Mate matrix. Adjustment can be made to the hydration fluid as follows:

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