Thomson Lab Protocols

Matrigel Aliquoting and Plating

Aliquoting Matrigel:

Day one:

- Put the sterilized tip box (either 200 ml or 1000 ml tips), sterilized microfuge tube container, and appropriate pipettor in -20 °C freezer.
- Thaw the Matrigel bottle on ice in the 4 °C fridge overnight (until it liquifies).

Day two:

- Each bottle of Matrigel is at a different density, and it is aliquoted out at 2mg/tube (which is enough to coat one six well plate).
- Fill an ice bucket and place the Matrigel, along with pipets and tubes on ice to keep them cold*.
  Example calculation with Matrigel arriving at 13.841 mg/ml:
  13.841mg/1ml = 2mg/x ml
  x = 0.145ml/tube
- Using good sterile technique, aliquot Matrigel to tubes, switching tips whenever Matrigel seems to be clogging the tip and/or causing the pipet to measure inaccurately. Place tubes on ice as they are finished.
- When the entire bottle has been aliquoted the tubes are stored in either the –20 °C or –70 °C until ready to be used.

How to Make Matrigel Plates:

There are at least two possible methods for this. The main objective is not to let the undiluted Matrigel sit at room temperature for too long (or it will become chunky and solidify).

- Thaw tube overnight on ice at 4 °C.
- Dilute with 6ml cold basal media and mix well.
- Add 1ml per well of 6 well plate.
- Allow plate to sit at room temperature for one hour or overnight at 4 °C.
- Plate may either be used immediately or stored at 4 °C (plate will be good for at least one week).
- When ready to use the plate(s), remove the excess liquid and wash once with basal medium.

A second possible method is a little quicker, the only modification being that you take a tube directly from the freezer and IMMEDIATELY resuspend the pellet of Matrigel in 6ml ice cold media. Keep pipetting vigorously until all chunks are gone, and add to plate.

If after either of these methods, your plate still looks like it has chunks of Matrigel instead of a smooth even layer, try placing the plate at 4 °C overnight. This should “re-melt” the Matrigel into an even layer. Some chunks are acceptable, but make sure that the whole surface of the plate is coated with Matrigel.

Caring for Cells Growing on Matrigel:

hES cells grown on Matrigel-coated plates require MEF-conditioned medium. This medium is identical to regular hES medium, but is made without bFGF. Twenty-four hours before using the medium, it is conditioned on MEFs (at a density of 2.12 × 105 cells/ml, 2.5ml/well of 6 well plate). bFGF is added fresh every time to each individual well (5ml/well).

*The main objective and constant throughout working with Matrigel is to keep it COLD. As Matrigel warms to room temperature, it begins to solidify, making it harder to work with (ie. sticking inside of the pipet) and making it chunkier when attempting to use and plate.