1. PURPOSE

To describe the thin coating method for tissue culture dishes and plates for the maintenance of iPSCs.

2. SUPPLIES

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix aliquot at desired concentration (Prepared in SOP-iPSC-001)

NOTE: A 0.5mg aliquot of Matrigel is re-suspended in 6mls of media and coats:

- Six wells of a 6-well plate (1ml/well)
- Twelve wells of a12-well plate (0.5ml/well)
- Twelve wells of 24-well plate (0.5ml/well)
- three 60mm dishes (2ml/dish)
- one 10cm dish
- one T-75 flask

Cold Basal media (DMEM, DMEM/F12, or F12)

Chilled 200ul sterile pipette tips

Chilled 15ml and 50ml sterile conical tubes

Chilled 5ml and 10ml sterile serological pipettes

3. SCOPE

This procedure applies to Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, *LDEV-Free for use as a substrate for iPSC culturing.

4. PROCEDURE

Day 0 - PREPARATION OF REAGENTS/SUPPLES

4.1 Place an appropriate amount of sterile P200 tips, sterile 15ml conical tubes (or 50ml conical tubes) and 5ml (or 10ml) sterile serological pipettes in the -80°C overnight.

NOTE: It is crucial that any item that will come in contact with the Matrigel be chilled. Matrigel will solidify and adhere to any item that is above 10°C.

Day 1 – MATRIGEL COATING

NOTE: The following steps must be performed in a sterile environment, such as a biosafety cabinet.

4.2 Calculate the concentration of Matrigel needed. Record your calculation on Reagent Table 1.1.

4.3 Calculate the volume of basal media needed. Record your calculation on Reagent Table 1.2.

Before beginning place your frozen serological pipettes, pipet tips, conical tubes and Matrigel aliquots on ice and place in BSC. Be sure to spray the ice bucket down thoroughly with 70% IPA before placing in the BSC.

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4.4 Add the volume of basal media calculated in step 4.3 into a cold conical using a room temperature pipette.

4.5 Using cold pipet tips take some volume of the cold basal media and add it directly to the frozen Matrigel aliquot

4.6 Gently pipet up and down to quickly thaw the aliquot.

NOTE: Take care to keep fingertips above the Matrigel level. The warmth from your fingertips will cause the Matrigel to solidify. Change to a new cold pipet tip frequently.

4.7 Transfer Matrigel mixture to the conical with cold media. Repeat until all Matrigel is added to cold media.

4.8 Using the cold serological pipette, transfer basal media/ Matrigel mixture to your TC dish(es) at the following volumes:

- 6-well plates = 1ml/well
- 12-well plates = 0.5ml/well
- 24-well plates = 0.5ml/well
- 60mm dishes = 2ml/dish
- 10cm dish = 6ml/dish
- T-75 flask = 6ml/flask

4.9 Swirl and/or rock the plate to ensure even coating.

4.10 Incubate at room temperature for at least 1 hour.

4.11 Alternatively, if you are not using Matrigel coated dishes on the same day as coating, wrap dishes in parafilm, and store in 4°C fridge for up to 1 week. **Make sure wells do not dry out**. A volume of basal media may be added to the well(s) 1 hour after coating to ensure that the wells do not dry out. If a portion of a well does dry out, **this well cannot be used**.

CAUTION: It is crucial that all items remain cold. **DO NOT** allow the Matrigel aliquots to warm to room temperature. Make sure to change pipette tips frequently to ensure that the tips touching the Matrigel are cold.

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REAGENTS TABLE 1.1 – CONCENTRATION OF MATRIGEL NEEDED

# of wells or plates		Calculation	Concentration of Matrigel Needed (ug)
# of 35mm wells (1 well of a 6 well plate)	X=	=X/12	
# of 12 well plates	X=	=X (0.5)	
# of 24 well plates	X=	=X (1.0)	
# of 60mm dishes (in multiples of 3)	X=	=X (0.5)	
# of 10cm dishes	X=	=X (0.5)	
# of T-75 flasks	X=	=X (0.5)	
Other	X=		
TOTAL CONCENTRATION OF MATRIGEL NEEDED =			ug

REAGENTS TABLE 1.2 – VOLUME OF BASAL MEDIA NEEDED

		Calculation (0.5mg = 6mls)	Volume of Basal Media Needed
Total Concentration of Matrigel from Table 1.1	X=	=X(12)	mls

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1. Troubleshooting

PROBLEM	POSSIBLE CAUSE	SOLUTION
While resuspending Matrigel the pipette tip clogged	Pipette tip has warmed to room temperature Matrigel has warmed to room temperature	Discard the clogged pipette tip and use a new chilled pipette tip Matrigel may be re-liquified if placed at 4°C in ice for 24-48 hours.
Center of the well has no Matrigel	The well has dried up Insufficient volume of media	Coat another well to use. **Do not use a well that has dried up**. Ensure that you are adding the correct volume of Media & Matrigel mixture to each well
Coated well looks “bumpy” after 1hr incubation	Matrigel solidified during the coating process	Plate can be placed in 4°C overnight

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