1. PURPOSE

To describe the procedure for preparing Matrigel aliquots used for tissue culture dish coating.

2. SUPPLIES

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, *LDEV-Free, 10mL (Product #354230)

Ice bucket
Chilled 0.5mL Eppendorf tubes
Chilled Eppendorf tube racks
Chilled 200uL Pipette tips
Chilled 1000uL Pipette tips

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/SUPPLIES

4.1 Thaw Matrigel® overnight by submerging the unopened bottle in an ice bucket filled with ice.

4.2 Place the lid on the ice bucket and store at 4°C overnight.

4.3 Acquire and appropriate amount of sterile 0.5mL Eppendorf tubes, tube racks, sterile 200uL pipette tips and 1000uL pipette tips and place 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 at room temperature.

Day 1 - MATRIGEL ALIQUOTS

**NOTE:** These steps must be performed in a sterile environment, such as a biosafety cabinet. Matrigel must be kept on ice at all times.

4.4 Match the lot # on the Matrigel bottle to the lot # on the specification sheet and note the concentration. Record below and in Reagents table 1.1:

| Lot #: _____________________ | Concentration: _____________________ |

4.5 Using the concentration provided on the specification sheet calculate the volume needed to obtain 0.5mg, 1mg and 2mg aliquots. Record your calculations in Reagents table 1.1.

4.6 Using the volumes obtained in step 4.5, calculate an appropriate number of 0.5mg’s, 1mg’s and 2mg’s that can be derived from 10mls of Matrigel.

4.7 Prepare Eppendorf tubes by placing opened pre-chilled tubes into a pre-chilled tube rack.

4.8 Open the Matrigel bottle by carefully removing the rubber stopper.

4.9 Using cold pipette tips, aliquot the appropriate volume of Matrigel into the cold tubes.
4.10 Label tubes and store at -20°C. Tubes may also be stored temporarily on ice while aliquots are being done.

**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. Change tubes racks frequently to keep Eppendorf tubes cold. Matrigel aliquots may also be performed on ice if cold tube racks are not available.

### REAGENTS TABLE 1.1 - MATRIGEL

<table>
<thead>
<tr>
<th>Lot #:</th>
<th>Concentration:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Desired mg)(1000ul)/[MG] = X uL</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume to obtain 0.5mg</td>
<td>(0.5mg)(1000ul)/__________ = ______ uL</td>
</tr>
<tr>
<td>Volume to obtain 1mg</td>
<td>(1mg)(1000ul)/__________ = ______ uL</td>
</tr>
<tr>
<td>Volume to obtain 2mg</td>
<td>(2mg)(1000ul)/__________ = ______ uL</td>
</tr>
</tbody>
</table>

### 5. Troubleshooting

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE</th>
<th>CAUSE SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clogged pipette tip</td>
<td>Pipette tip has warmed to room temperature</td>
<td>Discard the clogged pipette tip and use a new chilled pipette tip</td>
</tr>
<tr>
<td>Matrigel has warmed to room temperature</td>
<td>Matrigel may be re-liquified if placed at 4°C in ice for 24-48 hours.</td>
<td></td>
</tr>
<tr>
<td>Lot # on Matrigel bottle does not match the Lot # on the specification sheet</td>
<td>Looking at wrong specification sheet</td>
<td>Use correct specification sheet</td>
</tr>
<tr>
<td>Company sent the incorrect specification sheet</td>
<td>Check Corning website for correct spec sheet</td>
<td></td>
</tr>
</tbody>
</table>
1. PURPOSE

To describe the procedure for coating TC 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 at room temperature.

**Day 1 – MATRIGEL COATING**

**NOTE:** These steps must be performed in a sterile environment, such as a biosafety cabinet. These steps must be performed quickly to ensure that the Matrigel does not solidify.

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.

4.4 Acquire chilled conical tubes, 200ul pipette tips and serological pipettes from the -80°C freezer and place inside the hood.

4.5 Quickly add the volume of basal media calculated in step 4.3 into a cold conical using a room temperature pipette.

4.6 Grab the appropriate Matrigel aliquot(s) calculated in step 4.2 from the -20°C freezer.

4.7 Using the cold tips, quickly use cold media to thaw frozen Matrigel by gently pipetting up and down.
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 pipette tip frequently.

4.8 Transfer Matrigel to conical with cold media. Repeat until all Matrigel is added to cold media.

4.9 Using the cold serological pipette, transfer basal media/ Matrigel mixture to your TC dish(es).
   - 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

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

4.11 Incubate at room temperature for at least 1 hour.

4.12 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 PBS 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.

**REAGENTS TABLE 1.1 – CONCENTRATION OF MATRIGEL NEEDED**

<table>
<thead>
<tr>
<th># of wells or plates</th>
<th>Calculation</th>
<th>Concentration of Matrigel Needed (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td># of 35mm wells (1 well of a 6 well plate)</td>
<td>X=</td>
<td>=X/12</td>
</tr>
<tr>
<td># of 12 well plates</td>
<td>X=</td>
<td>=X (0.5)</td>
</tr>
<tr>
<td># of 24 well plates</td>
<td>X=</td>
<td>=X (1.0)</td>
</tr>
<tr>
<td># of 60mm dishes (in multiples of 3)</td>
<td>X=</td>
<td>=X (0.5)</td>
</tr>
<tr>
<td># of 10cm dishes</td>
<td>X=</td>
<td>=X (0.5)</td>
</tr>
<tr>
<td># of T-75 flasks</td>
<td>X=</td>
<td>=X (0.5)</td>
</tr>
<tr>
<td>Other</td>
<td>X=</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL CONCENTRATION OF MATRIGEL NEEDED</strong></td>
<td></td>
<td><strong>ug</strong></td>
</tr>
</tbody>
</table>
## REAGENTS TABLE 1.2 – VOLUME OF BASAL MEDIA NEEDED

<table>
<thead>
<tr>
<th>Total Concentration of Matrigel from Table 1.1</th>
<th>Calculation (0.5mg = 6mls)</th>
<th>Volume of Basal Media Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>X=</td>
<td>=X(12)</td>
<td>mls</td>
</tr>
</tbody>
</table>

## 5. Troubleshooting

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

To describe the procedure for manual passaging of iPSCs maintained on Matrigel using the StemPro EZ Passaging Tool.

2. SUPPLIES

- Complete mTeSR Medium (Basal medium + 5x Supplement) (StemCell Technologies, Cat # 05850)
- Matrigel Coated TC dish (Prepared as described in SOP-iPSC-002)
- StemPro® EZPassage™ Disposable Stem Cell Passaging Tool (Life Technologies, Cat # 23181-010)
- 5ml and 10ml sterile serological pipettes

3. PROCEDURE

**NOTE:** You must have a prepared Matrigel coated plate before starting this protocol. If you are using a Matrigel coated plate that has been stored at 4°C, the plate must be allowed to equilibrate to room temperature for 1 hour prior to starting.

3.1 Prior to passaging your cells, check colonies in a microscope and using a pulled glass pipette or colony marker, remove any areas of differentiation from the culture.

3.2 Aspirate spent media and add desired amount of fresh mTeSR + Supplement media to each well. For example: Add 3mls of media for 1:6 split (0.5ml of cells into 6 wells).

3.3 Aspirate Matrigel from the prepared Matrigel coated tissue culture plate and add 2mls of complete mTeSR media to each well (for a total of 2.5mls per well after cells have been added).

3.4 Using the EZPassage™ tool cut colonies into small squares.
   3.4.1 Hold the culture vessel in one hand and **pull (roll)** the StemPro® EZPassage™ Disposable Stem Cell Passaging Tool across the entire plate in one direction (Left to right). Apply enough pressure so the entire roller blade touches the plate and maintain uniform pressure during the rolling action.

3.4.2 Rotate the plate 90° and repeat step 3.4.1

**OPTIONAL:** View the plate in the microscope to visually confirm that the colonies have been properly cut. Colonies should have a “checkered” look to them.

3.5 Using 5ml serological pipette, aspirate medium from the well. Hold the serological tip perpendicular to the bottom of the well and then gently dislodge the colonies from the plate while simultaneously releasing medium.
NOTE: It is important to continuously have medium coming out of the pipette. Do not scrape the wells “dry” (no media). This will cause the cut colonies to smear and the cells will die.

3.6 Continue to pipette up and down gently to completely dislodge the cut colonies. Avoid causing bubbles.

3.7 Add 0.5ml of cell suspension to each well of the new 6-well plate.

3.8 Rock the plate back and forth and then side to side to ensure even distribution of colonies in the well.

3.9 Place the plate in 37°C incubator with 5% CO₂. Do not move the plate for 24 hours.

3.10 After 24 hours, view the plate in the microscope to confirm that the colonies have attached to the plate. Change medium.

4. Troubleshooting

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE</th>
<th>CAUSE SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies are still whole after rolling EZ Pass tool over them</td>
<td>Did not exert the proper amount of pressure when rolling the tool over the well</td>
<td>Place a greater amount of pressure onto the tool (similar to the amount of pressure you would place on a pen when writing)</td>
</tr>
<tr>
<td></td>
<td>The EZ pass tool is not touching the bottom of the well</td>
<td>Ensure that the EZ Pass tool is touching the bottom of the well. You may have to adjust the angle at which the tool is entering the well</td>
</tr>
<tr>
<td>Colonies appear smeared after rolling the EZ Pass tool over them</td>
<td>Too much pressure was applied to the EZ Pass tool when rolling the tool over the well</td>
<td>Use less pressure when using the tool (similar to the amount of pressure you would place on a pen when writing)</td>
</tr>
<tr>
<td>Colonies are still stuck to the bottom of the plate</td>
<td>Did not scrape the colonies properly with a serological pipette</td>
<td>Repeat step 3.5, applying more pressure when scraping the cells</td>
</tr>
<tr>
<td>Colonies appear smeared after scraping the wells (step 3.5)</td>
<td>Did not have medium coming out from the pipette while scraping</td>
<td>These cells are unusable. Perform the protocol on a different well of cells. Make sure to continuously be releasing medium from the pipette during the scraping process</td>
</tr>
<tr>
<td>Colonies have all attached to the center of the plate</td>
<td>Colonies did not evenly spread across the plate</td>
<td>Make sure to perform step 3.8. Perform this step right before placing the plate into the incubator</td>
</tr>
<tr>
<td>Colonies did not attach to the plate after passaging</td>
<td>Matrigel plates were old or died out</td>
<td>Ensure that you are using a Matrigel plate that is no more than 1 week old and that every well is properly coated</td>
</tr>
<tr>
<td></td>
<td>Cell line may have difficulties attaching</td>
<td>Cell line may require a double concentration of Matrigel. Coat a new plate.</td>
</tr>
</tbody>
</table>
1. PURPOSE
To describe the procedure for chemical passaging of iPSCs maintained on Matrigel using Versene

2. SUPPLIES
Complete mTeSR Medium (Basal medium + 5x Supplement) (StemCell Technologies, Cat # 05850)
Matrigel Coated TC dish (Prepared as described in SOP-iPSC-002)
Versene (Life Technologies, Cat # 15040-066)
5ml and 10ml sterile serological pipettes

3. PROCEDURE
**NOTE:** You must have a prepared Matrigel coated plate before starting this protocol. If you are using a Matrigel coated plate that has been stored at 4°C, the plate must be allowed to equilibrate to room temperature for 1 hour prior to starting.

3.1 Prior to passaging your cells, check colonies in a microscope and using a pulled glass pipette or colony marker, remove any areas of differentiation from the culture.

3.2 Aspirate Matrigel from the prepared Matrigel coated tissue culture plate and add 2mls of complete mTeSR media to each well (for a total of 2.5mls per well after cells have been added).

3.3 Aspirate spent media.

3.4 Rinse the wells with one volume of Versene and aspirate
   3.4.1 Use 1ml for a single well of 6-well plate
   3.4.2 Use 1.5ml for a single 60mm dish
   3.4.3 Use 0.5ml for a single well of a 12-well dish

3.5 Add a volume of Versene to each well.

3.6 Incubate at 37°C for 4-5 minutes.
   **Optional:** Check cells under microscope after 4-5 minutes to check for the breaking up of colonies.

3.7 Gently rinse the wells with a single volume of mTeSR.
   **NOTE:** Typically, the cells should NOT lift from the plate at this point. You will lose a minimal amount of cells. **If too many cells have lifted off the plate,** add additional media to the well and collect the cells into a sterile 15ml conical. Centrifuge the conical for 1 minute at 1000rpm. Proceed to step 3.8.

3.8 Add an appropriate volume of mTeSR and pipette up and down to dislodge the cells.
NOTE: If you have collected and centrifuged your cells, you will use this step to break up your cell pellet.

3.9 Pass cells at desired density into a new Matrigel coated TC dish.

EXAMPLE: Add 3mls of mTeSR at step 3.8, then distribute 0.5ml of cell suspension to each well for a 1:6 split.

3.10 Rock the plate back and forth and then side to side to ensure even distribution of colonies in the well.

3.11 Place the plate in 37°C incubator with 5% CO₂. Do not move the plate for 24 hours.

3.12 After 24 hours, view the plate in the microscope to confirm that the colonies have attached to the plate. Change medium.

4. Troubleshooting

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE</th>
<th>CAUSE SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies are still whole after 5 minute incubation</td>
<td>Cells may need a longer incubation period</td>
<td>Place the plate back into the incubator and check for colony break up every 3 minutes</td>
</tr>
<tr>
<td>Colonies will not dislodge after 5 minute incubation</td>
<td>Cells may need a longer incubation period</td>
<td>Place the plate back into the incubator and check for colony break up every 3 minutes</td>
</tr>
<tr>
<td>Colonies have all attached to the center of the plate</td>
<td>Colonies did not evenly spread across the plate</td>
<td>Make sure to perform step 3.10. Perform this step right before placing the plate into the incubator</td>
</tr>
<tr>
<td>Colonies did not attach to the plate after passaging</td>
<td>Matrigel plates were old or died out</td>
<td>Ensure that you are using a Matrigel plate that is no more than 1 week old and that every well is properly coated</td>
</tr>
<tr>
<td></td>
<td>Cell line may have difficulties attaching</td>
<td>Cell line may require a double concentration of Matrigel. Coat a new plate.</td>
</tr>
</tbody>
</table>
1. PURPOSE
   To describe the procedure for freezing iPSC colonies for cryopreservation.

2. SUPPLIES
   Complete mTeSR Medium (Basal Medium + 5x Supplement)
   CryoStor CS10 (Stemcell Technologies, Cat # 07930)
   5ml and 10ml sterile serological pipettes
   Nalgene Cryovials (Fisher Scientific, Cat # 03-337-7Y)
   BD Falcon Cell Scraper (VWR, Cat # 15621-005)
   Sterile 15ml conical tube

3. PROCEDURE
   3.1 Prior to freezing your cells, check colonies in a microscope and using a pulled glass pipette or colony marker, remove any areas of differentiation from the culture.
   3.2 Aspirate spent media.
   3.3 Add 1ml of fresh mTeSR to each well.
   3.4 Using a cell scraper, gently lift the colonies from the plate.
      **NOTE:** It is important that you do not exert too much pressure when using the cell scraper. Too much pressure can cause the cell scraper to “smash” or smear the colonies, rendering them unusable.
   3.5 Collect the cells in a sterile 15ml conical.
   3.6 Centrifuge the cells for 1 minute at 1000rpm
      **Optional:** You may also allow the cells to settle via gravity by standing the conical tube upright for 5-7 minutes.
   3.7 Aspirate the supernatant without disturbing the cell pellet.
   3.8 Re-suspend the cell pellet in an appropriate volume of CryoStor CS10 to obtain 1ml per cryovial.
      **NOTE:** Typically, one confluent well of a 6-well dish can be distributed into 2 cryovials.
   3.9 Rock the plate back and forth and then side to side to ensure even distribution of colonies in the well.
   3.10 Add cells to cryovials and freeze using an isopropanol freezing vessel at -80°C overnight.
   3.11 Transfer frozen vials to an LN2 tank.
# THAWING IPSCS FOR MAINTANENCE AND EXPANSION

## SOP Number: SOP-iPSC-006 | Version: A

<table>
<thead>
<tr>
<th>STANDAR OPERATING PROCEDURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDUCED PLURIPOTENT STEM CELL CORE</td>
</tr>
<tr>
<td>THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY</td>
</tr>
</tbody>
</table>

## 1. PURPOSE

To describe the procedure for thawing iPSC colonies for maintenance and expansion.

## 2. SUPPLIES

- Complete mTeSR Medium (Basal Medium + 5x Supplement)
- Matrigel Coated TC dish (Prepared as described in SOP-iPSC-002)
- 5ml and 10ml sterile serological pipettes
- Sterile 15ml conical tube

## 3. PROCEDURE

**NOTE:** You must have a prepared Matrigel coated plate before starting this protocol. If you are using a Matrigel coated plate that has been stored at 4°C, **the plate must be allowed to equilibrate to room temperature for 1 hour prior to starting.**

3.1 Remove cells from the LN2 tank.

3.2 Thaw cells quickly in a 37°C water bath using a “figure 8” motion until you see a pea sized ball of ice.

3.3 Using a 2ml pipette, move frozen cells into a sterile 15ml conical

3.4 Slowly add mTeSR medium to conical drop by drop to dilute CryoStor CS10 (1:10 ratio is recommended).

3.5 Centrifuge the conical/cell mixture for 1 minute at 1000rpm.

3.6 While cells are spinning, aspirate Matrigel from dish and add an appropriate volume of mTeSR to the well.

3.7 Aspirate the medium from cells and re-suspend cells to desired volume with mTeSR.

3.8 Plate the cells into the new well.

**NOTE:** 1 cryovial will typically thaw into one well of a 6-well plate.

3.9 Rock the plate back and forth and then side to side to ensure even distribution of colonies in the well.

3.10 Place the plate in a 37°C incubator with 5% CO₂. Do not move the plate for 24 hours.

3.11 After 24 hours, view the plate in the microscope to confirm that the colonies have attached to the plate. Change medium.