## 1. PURPOSE

To describe the procedure for preparing single use only Matrigel aliquots used for thin coating of tissue culture dish.

## 2. SUPPLIES

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

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## 4. PROCEDURE

**Day 0 - PREPARATION OF REAGENTS/SUPPLES**

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 above 10°C.

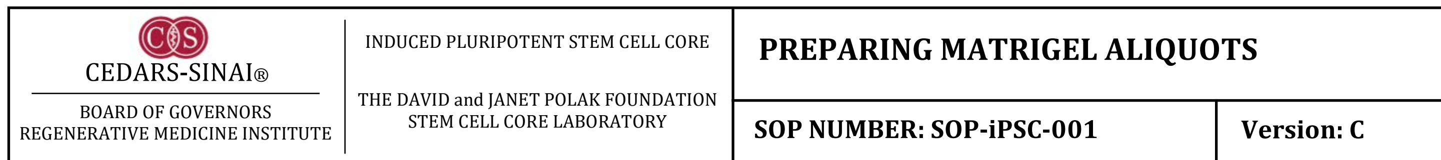
**Day 1 - MATRIGEL ALIQUOTS**

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

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 Swirl bottle to ensure that material is evenly dispersed.

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

4.10 Using cold pipette tips, aliquot the appropriate volume of Matrigel into the cold tubes.

4.11 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. Making Matrigel aliquots may also be performed on ice if cold tube racks are not available.

**REAGENTS TABLE 1.1 - MATRIGEL**

| Lot #:<br>Concentration: | | |
|---|---|---|
| | (Desired mg)(1000ul)/ [MG] = X uL | # of Tubes |
| Volume to obtain 0.5mg | (0.5mg)(1000ul)/________ = ______ uL | |
| Volume to obtain 1mg | (1mg)(1000ul)/________ = ______ uL | |
| Volume to obtain 2mg | (2mg)(1000ul)/________ = ______ uL | |

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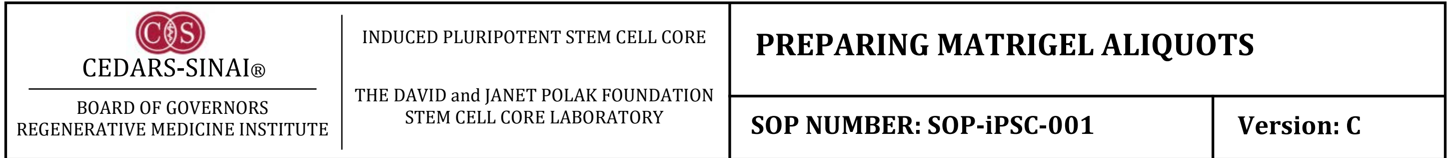


## 5. Troubleshooting

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

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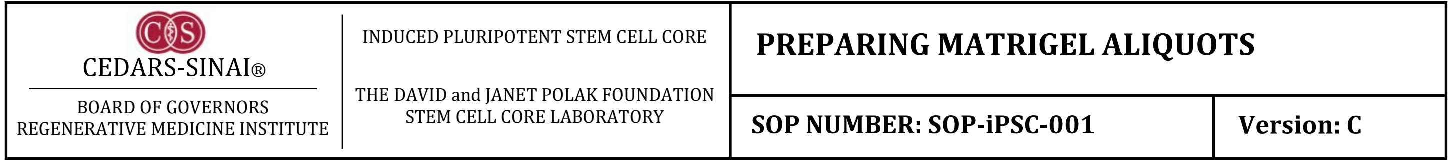




# Matrigel Aliquots

Catalog Number: ____________________

Lot Number: ____________________

Concentration: ___________mg/ml___

Protocol used: _SOP-iPSC-001

Type of Aliquot: __________mg_______

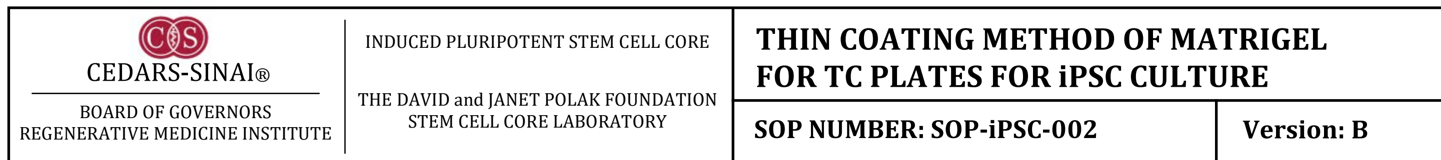
Number of Aliquots: _____________

Label (from bottle):

Aliquots made by: ________________________________

Date made: _____________________________

# STANDARD OPERATING PROCEDURE



| CEDARS-SINAI® BOARD OF GOVERNORS REGENERATIVE MEDICINE INSTITUTE | INDUCED PLURIPOTENT STEM CELL CORE<br>THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY | **THIN COATING METHOD OF MATRIGEL FOR TC PLATES FOR iPSC CULTURE** | |
|---|---|---|---|
| | | **SOP NUMBER: SOP-iPSC-002** | **Version: B** |

## 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**

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.

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

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

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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**.

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**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

| # 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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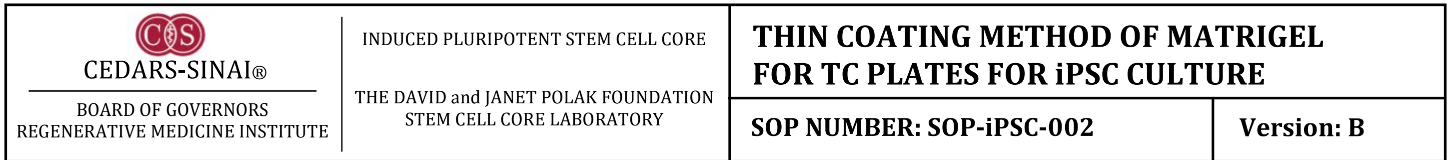


## 5. Troubleshooting

| PROBLEM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| While resuspending Matrigel the pipette tip clogged | Pipette tip has warmed to room temperature<br><br>Matrigel has warmed to room temperature | Discard the clogged pipette tip and use a new chilled pipette tip<br><br>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<br><br>Insufficient volume of media | Coat another well to use. **Do not use a well that has dried up**.<br><br>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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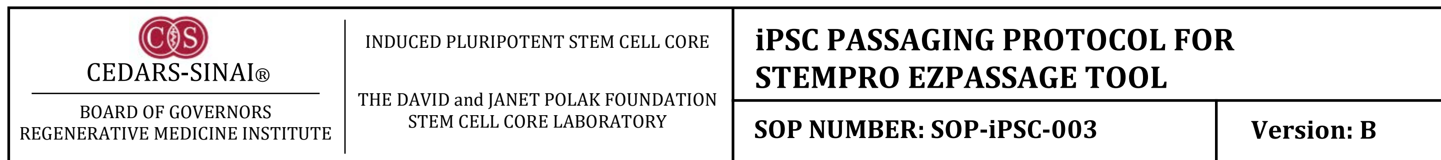
# STANDARD OPERATING PROCEDURE




## 1. PURPOSE

To describe the procedure for manual passaging of iPSCs maintained on Matrigel using the StemPro EZ Passage 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 $4^{o}$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 cleaning tool to 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 final volume 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™ 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 Continue to pull the tool parallel until you have covered the entire plate in one direction.

Note: Do not move the tool back and forth throughout the well; only move in the left to right direction.

3.4.3 Rotate the plate $90^{o}$ 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.

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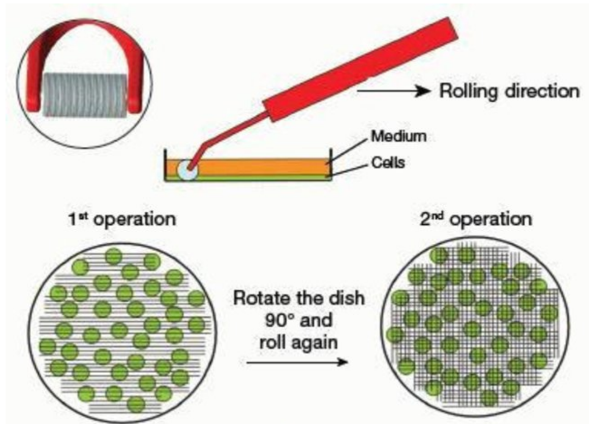
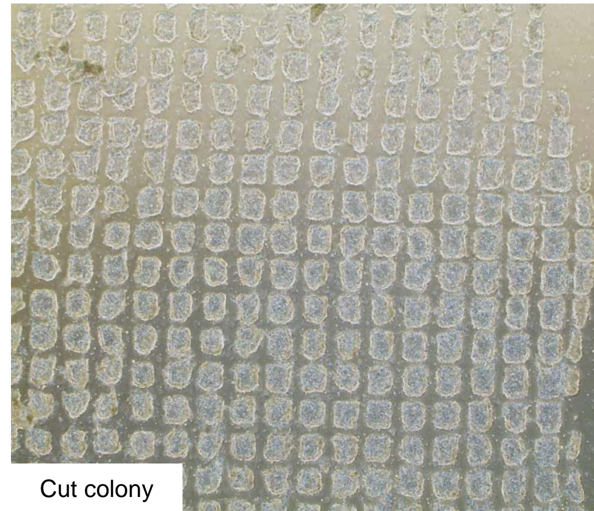



Figure 2. Procedure for cutting hESC/hiPSC colonies using the StemPro® EZPassage™ tool. Tiny grooves in the StemPro® EZPassage™ tool facilitate cutting of embryonic stem cell colonies.




3.5 Using 5ml serological pipette, pull up the media from the well.

3.6 Hold the serological tip perpendicular to the bottom of the well and then gently scrape the well to 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.

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

3.7.1 Try not to repeat steps 3.5 – 3.7 more than 5 times because this will cause more cell death because it will break up the colony sizes too small.

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

3.9 Place the plate in the 37°C incubator with 5% $CO_2$ and gently rock the plate back and forth and side-to-side to ensure even distribution of the colonies throughout the well.

3.10 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.

3.12 Change the media every day until ready to be used or passaged again.

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## 4. Troubleshooting

| PROBLEM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| Colonies are still whole after rolling EZ Pass tool over them | Did not exert the proper amount of pressure when rolling the tool over the well<br><br>The EZ pass tool is not touching the bottom of the well | Place a greater amount of pressure onto the tool (like the amount of pressure you would place on a pen when writing)<br>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 |
| Colonies appear smeared after rolling the EZ Pass tool over them | Too much pressure was applied to the EZ Pass tool when rolling the tool over the well | Use less pressure when using the tool (like the amount of pressure you would place on a pen when writing). The handle of the tool should not bend while in use |
| Colonies are still stuck to the bottom of the plate | Did not scrape the colonies properly with a serological pipette | Repeat step 3.5 – 3.7, applying more pressure when scraping the cells |
| Colonies appear smeared after scraping the wells (step 3.6) | Did not have medium coming out from the pipette while scraping | 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 |
| Colonies have all attached to the center of the plate | Colonies did not evenly spread across the plate | Make sure to perform step 3.9. Perform this step in the incubator so after the plate is rocked back and forth it won't be disturbed<br>Place plates in the back of the incubator to ensure they are not bumped after passing<br>Open and close the incubator door gently to prevent any dislodging |
| Colonies did not attach to the plate after passaging | Matrigel plates were old or died out<br><br>Cell line may have difficulties attaching | Ensure that you are using a Matrigel plate that is no more than 2 weeks old and that every well is properly coated<br>Cell line may require a double concentration of Matrigel. Coat a new plate. |

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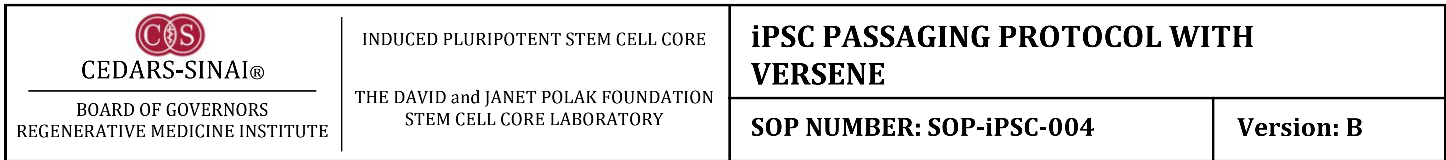
# STANDARD OPERATING PROCEDURE



| CEDARS-SINAI® BOARD OF GOVERNORS REGENERATIVE MEDICINE INSTITUTE | INDUCED PLURIPOTENT STEM CELL CORE<br>THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY | **iPSC PASSAGING PROTOCOL WITH VERSENE** | |
|---|---|---|---|
| | | **SOP NUMBER: SOP-iPSC-004** | **Version: B** |

## 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 $4^{\circ}$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 cleaning tool 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 $37^{\circ}$C for 4-5 minutes.

**Optional:** Check cells under microscope after 4-5 minutes to check for the breaking up of colonies.

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3.7 Aspirate Versene and 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 number 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 Place the plate in the 37°C incubator with 5% $CO_2$ and gently rock the plate back and forth and side-to-side to ensure even distribution of the colonies throughout the well.

3.11 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.

3.13 Change the media every day until ready to be used or passaged again.

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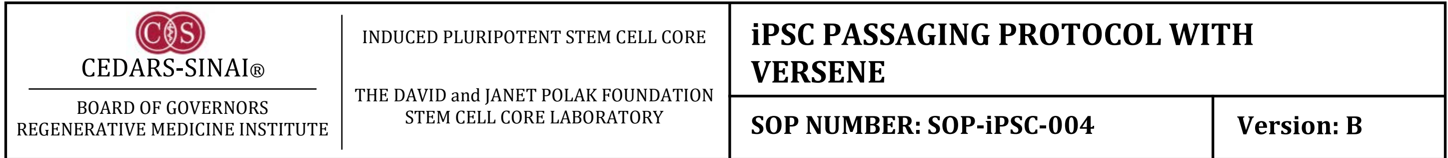


## 4. Troubleshooting

| PROBLEM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| Colonies are still whole after 5-minute incubation | Cells may need a longer incubation period | Place the plate back into the incubator and check for colony break up every 3 minutes |
| Colonies will not dislodge after 5-minute incubation | Cells may need a longer incubation period | Place the plate back into the incubator and check for colony break up every 3 minutes |
| Colonies have all attached to the center of the plate | Colonies did not evenly spread across the plate | Make sure to perform step 3.10. Perform this step in the incubator so after the plate is rocked back and forth it won't be disturbed<br>Place plates in the back of the incubator to ensure they are not bumped after passing<br>Open and close the incubator door gently to prevent any dislodging |
| Colonies did not attach to the plate after passaging | Matrigel plates were old or died out<br><br>Cell line may have difficulties attaching | Ensure that you are using a Matrigel plate that is no more than 1 week old and that every well is properly coated<br>Cell line may require a double concentration of Matrigel. Coat a new plate. |

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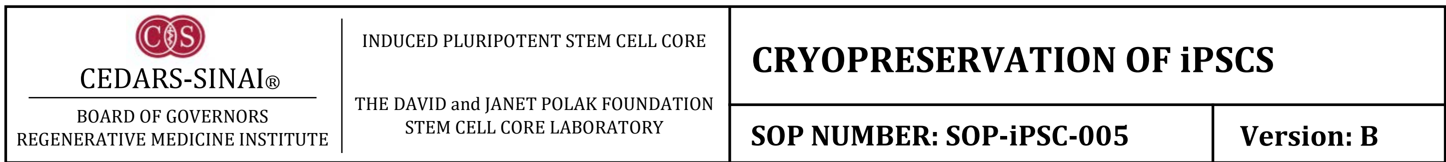
# STANDARD OPERATING PROCEDURE




| INDUCED PLURIPOTENT STEM CELL CORE<br>THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY | **CRYOPRESERVATION OF iPSCS** | |
|---|---|---|
| | **SOP NUMBER: SOP-iPSC-005** | **Version: B** |

## 1. PURPOSE

To describe the procedure for freezing iPSC colonies for cryopreservation.

## 2. SUPPLIES

Complete mTeSR Medium (Basal Medium + 5x Supplement) (StemCell Technologies, Cat #85850)

CryoStor CS10 (StemCell Technologies, Cat #07930)

5ml and 10ml sterile serological pipettes

Thermo Scientific™ Nalgene™ General Long-Term Storage Cryogenic Tubes (Fisher Scientific, Cat #03-337-7Y)

Corning™ Falcon™ Cell Scraper (Fisher Scientific, Cat #08-771-1A)

Sterile 15ml conical tube

## 3. PROCEDURE

3.1 Prior to freezing your cells, check colonies in a microscope and using a cleaning tool, 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 and pipet up and down 3 – 4 times to break up colonies

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.

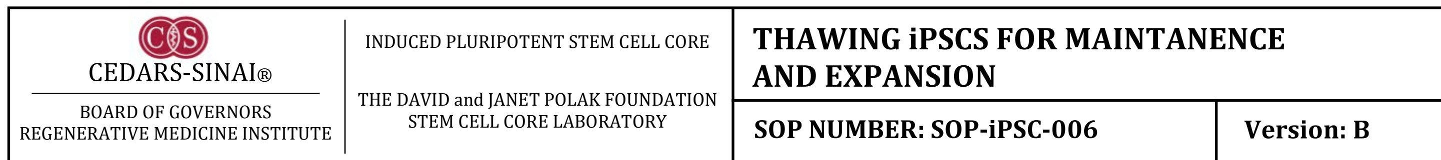
3.7 Aspirate the supernatant without disturbing the cell pellet

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 plate can be distributed into 2 cryovials.

3.9 Add cells to cryovials and freeze using an isopropanol freezing vessel at -80°C overnight

3.10 Transfer frozen vials to an $LN_2$ tank

# STANDARD OPERATING PROCEDURE



| CEDARS-SINAI® BOARD OF GOVERNORS REGENERATIVE MEDICINE INSTITUTE | INDUCED PLURIPOTENT STEM CELL CORE THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY | **THAWING iPSCS FOR MAINTANENCE AND EXPANSION** | |
|---|---|---|---|
| | | **SOP NUMBER: SOP-iPSC-006** | **Version: B** |

## 1. PURPOSE

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

## 2. SUPPLIES

Complete mTeSR Medium (Basal Medium + 5x Supplement) (StemCell Technologies, Cat #85850)

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**.

**NOTE: 1 cryovial should be thawed into 1 well of a 6 well plate**

3.1 Add 9mls of cold mTeSR medium to a sterile 15ml conical.

NOTE: A 1:10 ratio is recommended to effectively dilute the Cryostor CS10 (1ml of cells and 9mls of mTeSR).

3.2 Remove cells from the $LN_2$ tank.

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

3.4 Using a 2ml pipette, slowly add cells to the 9mls of mTeSR drop by drop.

3.5 Cap the conical and gently invert the tube 4-5 times to mix the CryoStor CS10 and mTeSR.

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

3.7 While cells are spinning, aspirate Matrigel from dish and add an 1ml of mTeSR to the well (for a final volume of 2.5mls per well after cells have been added).

3.8 Aspirate the medium from cells and re-suspend cells in 1.5mls of fresh mTeSR.

3.9 Plate the cells into the new well.

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

3.10 Place the plate in the 37°C incubator with 5% $CO_2$ and gently rock the plate back and forth and side-to-side to ensure even distribution of the colonies throughout the well.

3.11 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.

3.12.1 If there is very little attachment the day after thawing, don't change the media until the next day to give the cells more time to settle and attach firmly.

3.13 Change the media every day until ready to be used or passaged.

CEDARS-SINAI REGENERATIVE MEDICINE INSTITUTE INDUCED PLURIPOTENT STEM CELL CORE