


# STANDARD OPERATING PROCEDURE

|   |   |                                    |                   |
|---|---|------------------------------------|-------------------|
|  <b>Cedars<br/>Sinai</b> | INDUCED PLURIPOTENT STEM CELL CORE                                | <b>PREPARING MATRIGEL ALIQUOTS</b> |                   |
|   | THE DAVID and JANET POLAK FOUNDATION<br>STEM CELL CORE LABORATORY | <b>SOP NUMBER: SOP-iPSC-001</b>    | <b>Version: C</b> |

## 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.

## 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.

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- 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 #:                 |  |            |
| Concentration:         |  |            |
|                        | $(\text{Desired mg})(1000\text{ul}) / [\text{MG}] = \text{X uL}$                                 | # of Tubes |
| Volume to obtain 0.5mg | $(0.5\text{mg})(1000\text{ul}) / \underline{\hspace{1cm}} = \underline{\hspace{1cm}} \text{ uL}$ |            |
| Volume to obtain 1mg   | $(1\text{mg})(1000\text{ul}) / \underline{\hspace{1cm}} = \underline{\hspace{1cm}} \text{ uL}$   |            |
| Volume to obtain 2mg   | $(2\text{mg})(1000\text{ul}) / \underline{\hspace{1cm}} = \underline{\hspace{1cm}} \text{ uL}$   |            |

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### 5. Troubleshooting

| PROBLEM  | POSSIBLE                                       | CAUSE SOLUTION  |
|--|--|---|
| Clogged pipette tip  | Pipette tip has warmed to room temperature     | Discard the clogged pipette tip and use a new chilled pipette tip     |
|  | Matrigel has warmed to room temperature        | 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           | Use correct specification sheet                                       |
|  | Company sent the incorrect specification sheet | Check <a href="#">Corning website</a> for correct spec sheet          |




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## Matrigel Aliquots

Catalog Number: \_\_\_\_\_

Lot Number: \_\_\_\_\_

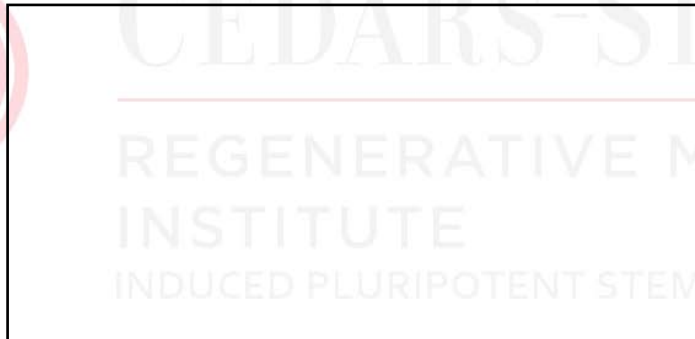
Concentration: \_\_\_\_\_ mg/ml

Protocol used: SOP-iPSC-001

Type of Aliquot: \_\_\_\_\_ mg

Number of Aliquots: \_\_\_\_\_

Label (from bottle):



*(Faint background watermark text: CEDARS SINAI REGENERATIVE MEDICINE INSTITUTE INDUCED PLURIPOTENT STEM CELL CORE)*

Aliquots made by: \_\_\_\_\_

Date made: \_\_\_\_\_