

STANDARD OPERATING PROCEDURE

 CEDARS-SINAI BOARD OF GOVERNORS REGENERATIVE MEDICINE INSTITUTE	INDUCED PLURIPOTENT STEM CELL CORE	PREPARING MATRIGEL ALIQUOTS	
	THE DAVID and JANET POLAK FOUNDATION STEM CELL CORE LABORATORY	SOP Number: SOP-iPSC-001	Version: A

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/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 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.

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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

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

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 Corning website for correct spec sheet