SAN DIEGO NATHAN SHOCK CENTER



PREPARING MATRIGEL COATED PLATES

SD-NSC Human Cell Models of Aging

Composed: A. Pankonin and K. Diffenderfer August 2020

Published: INSERT DATE

Research reported in this publication was supported by the National Institute On Aging of the National Institutes of Health under Award Number P30AG068635

WWW.SALK.EDU

Matrigel is used to culture hPSCs in feeder-free conditions



- Matrigel is an undefined basal membrane extract (BME) harvested from Engelbreth-Holm-Swarm mouse sarcomas
- While the composition of Matrigel and equivalent BMEs is undefined, these substrates are primarily composed of laminin, collagen IV, entactin, and heparin sulfate
- Matrigel readily polymerizes to form a 3D hydrogel at room temperature. It is crucial to <u>keep reagents cold</u> throughout the plating process to ensure proper coating
- 1mg of Matrigel is used to coat two welled plates or two 10cm dishes
- If using large format flasks or dishes assume 1mg/110cm²

There are other options and don't forget to lot test!

- Sak Where cures begin.
- Several manufacturers offer a growth factor reduced basal membrane extract product equivalent to Matrigel (Corning). Cultrex BME (R&D Systems) and Geltrex (ThermoFisher) are also commonly used.
- The Salk Stem Cell Core historically used Matrigel (Cat No. 354230). Since 2016 we've exclusively used Cultrex BME Type 1 (Cat No. 3433-005-01).
- As Matrigel and other BMEs are an undefined biological product lot testing is encouraged to assess quality of individual lots prior to purchase.



Gather your materials







- Ice bucket and ice
- Matrigel aliquot
- DMEM-F12, cold
- 50mL Tube
- 6-well plate (or other culture plates/flasks)

Label plates and prepare hood



- Label and date the side of your plates
- Gather your materials, including a P1000 micropipette and tips



Note: It is important to date plates as old Matrigel can lead to cell attachment and growth issues.

Resuspend matrigel in cold DMEM-F12





- Transfer appropriate volume of <u>cold</u>
 DMEM-F12 into a 50mL tube
- Resuspend Matrigel with a small volume of cold DMEM-F12 (~500-750uL) using a P1000.
- Transfer the Matrigel solution to tube of cold DMEM-F12
- Rinse Matrigel tube with ~1mL DMEM-F12/Matrigel solution to ensure all material is collected

Note: Keep everything on ice! All the reagents must be kept cold

Mix and plate matrigel to culture-ware



- Gently mix matrigel solution before plating
- Add 2mL matrigel solution to each well. Pipette carefully to ensure even plating.
- Incubate plates overnight at 37C before use.
- Store coated plates at 37C and use within 5 days



Note: If needed urgently incubate at 37C for \geq 3 hrs – longer is always better! We recommend fresh matrigel plates (\leq 3 days old) for more sensitive applications (thawing/low density plating).