Matrigel Coating Procedure for Feeder Free Culture of Human Pluripotent Stem Cells

Purpose: This protocol describes the Matrigel coating process for the purpose of maintaining human pluripotent stem cells (hPSC) in feeder-free conditions. Matrigel is an undefined basal membrane extract (BME) harvested from Engelbreth-Holm-Swarm mouse sarcoma cells. While the composition of Matrigel and equivalent BMEs are undefined, these substrates are primarily composed of laminin, collagen IV, entactin, and heparin sulfate. As Matrigel readily polymerizes to form a 3D hydrogel at room temperature, it is crucial to keep reagents cold throughout the plating process to ensure proper coating. 1mg of Matrigel is used to coat two welled plates (coating for 2x 6-well plates is described here) or two 10cm dishes. If using large format flasks or dishes, assume 1mg/110cm2.

Materials:

- Ice bucket and ice
- Matrigel aliquot (1mg, 2mg, and 4mg sizes are available)
- DMEM-F12 (cold)
- 50mL tube
- 6 wells plates (or other culture plates/flasks)

Procedure:

1. Gather a 1mg aliquot of Matrigel from the -80C freezer and place on ice.
2. Aliquot 24mLs of cold DMEM-F12 into a 50mL tube.
3. Using a P1000 collect 1mL of cold DMEM-F12 from the 50mL tube. Use the 1mL of DMEM-F12 to completely resuspend the Matrigel aliquot.
4. Transfer the resuspended Matrigel solution to the 50mL tube. Pipette up and down several times to rinse out the tip.
5. With a 25mL pipette mix the Matrigel solution, collect, and seed 2mL into each well.
6. Incubate Matrigel plates overnight at 37°C before use. An overnight incubation is recommended for best results. If an overnight incubation is not feasible incubate plates at 37C for at least 3 hours.
7. Coated plates can be stored at 37°C for up to 7 days before use.

Notes: Several manufacturers offer a basal membrane extract product equivalent to Matrigel. The Salk Stem Cell Core has had good success with Corning's growth factor reduced Matrigel product (Cat No. 354230), as well as, R&D's growth factor reduced Cultrex BME product (Cat No. 3433-005-01). The Salk Institute Stem Cell Core facility now exclusively stocks the R&D product. As Matrigel and other BMEs are an undefined biological product lot testing is encouraged to assess quality of individual lots prior to purchase.

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