Humboldt-CIRM Shared Resource Lab	STANDARD OPERATING PROCEDURE

Title: Aliquoting, Diluting, and Coating with hESC-qualified Matrigel
Purpose: To describe standard procedure handling Matrigel
Version: 1
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Materials and Supplies

Item	Purpose	Storage	Cat #
Matrigel (hESC-Qualified)	Basement membrane	-20°C	Corning 354277
KnockOut DMEM	Diluent for Matrigel aliquots	2-8°C	Thermo 10829018
1.5 ml Tubes	Storage of aliquots	RT	
Filter Pipette Tips	Liquid handling	RT	
Cooling Block	Maintaining low temperature	RT / -80°C	
Bucket of Ice	Prepared bed of crushed ice for handling		

Aliquoting Matrigel

This protocol is designed specifically for the standard expansion and maintenance of undifferentiated hPSCs using hESC-Qualified Matrigel. Matrigel was originally derived from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma. While the tumor's ability to produce a basement membrane was first described by Orkin, Martin, and colleagues in 1977 [(1)], the matrix was later developed into the standardized laboratory tool known as Matrigel by Hynda Kleinman and her team at the NIH [(2)]. **Note:** If you will be transitioning from expansion to differentiation, consider switching to Growth Factor Reduced (GFR) Matrigel which contains reduced concentrations of growth factors (e.g., TGFβ and EGF) that otherwise might affect the differentiation [(3-5)].

1. **Thawing:** Thaw a bottle of Matrigel overnight on ice in the back of a 4°C refrigerator, in order to prevent exposure to temperature fluctuations due to opening and closing.

2. **Calculating aliquot volumes:** check the lot-specific Certificate of Analysis (CoA) for the dilution volume. This is the amount of Matrigel required for 25 mL of KnockOut (KO) DMEM. (Lot-specific dilution). **Note:** For lower usage rates, you can pipette 50% of the CoA volume per tube to be suspended in 12.5 mL of KO DMEM (e.g., if the lot-specific volume is 270 µL, pipette 135 µL per tube) and then resuspending that half-aliquot in 12.5 mL of KO DMEM.

3. **Pre-Chill Plasticware:** Place the predetermined number of sterile 1.5 ml microcentrifuge tubes in a cooling block, along with the appropriate pipette tips, the in the -80°C freezer for at least 1 hour before use.

4. **Remove chilled materials**:

    a. Transfer the pre-chilled cooling block (with tubes) onto a prepared bed of crushed ice inside the TC hood. Sanitize both the block and the ice bed with 70% EtOH, wipe dry, and then open the tube lids.

    b. Remove the Matrigel from the 4°C, spray with 70% EtOH, wipe dry. Carefully remove the metal band and stopper, then place the bottle securely into the ice bucket in a stable location.

    c. Remove chilled tip box from the -80°C, spray with 70% EtOH, place in the ice box.

5. **Aliquot:** Immediately begin to aliquot the Matrigel into the pre-chilled tubes, aiming to complete the entire process within 5 -10 minutes. Use a new pipette tip every few aliquots as they will begin to warm and can cause the Matrigel to solidify. Close the lids.

6. **Storage:** Upon completion, immediately transfer the aliquots to a -80 (preferably towards the rear) avoid repeated freeze-thaw cycles.

Dilution and Coating

1. **Thawing**: Remove one aliquot of Matrigel from the -80°C freezer, immediately place on ice, and into the back of a 4°C refrigerator to thaw overnight.

2. **Resuspension:** Once thawed, spray the aliquot with 70% EtOH, dry the tube and then immediately resuspend the Matrigel aliquot into an appropriate, predetermined volume of cold KnockOut DMEM.

3. **Coating:** Add 1 ml of the solution per well of a 6-well plate. It is critical to make sure that the entire surface area of the well or vessel is covered. Different volumes will be required for different culture vessels (for information about approximate volume conversions click here). Any remaining Matrigel/KO-DMEM solution can be stored for up to 2 weeks in the back of the 4°C refrigerator.

4. **Incubation:** Incubate the coated dish at 37°C for ≥ 1 hour or overnight at 4°C, before use.

Troubleshooting:

Problem	Possible Cause	Solution
Tips are clogging or Matrigel is solidifying	Tips or Matrigel are warming to room temperature.	Change pipette tips every few aliquots; keep the Matrigel bottle securely in a stable ice bucket.
Well surface is not fully coated	Insufficient volume or the solution was not spread evenly.	Ensure the entire surface area of the vessel is covered. Use 1 ml of solution per well for a 6-well plate. Discard any plate not fully coated.
Poor cell attachment	Well surface not fully coated, Matrigel is old, dried out, or improperly stored.	Ensure coated plates are used within 2 weeks. Seal plates (e.g., with Parafilm) to prevent evaporation during 4°C storage.

Further Reading:

1. Orkin RW, et al. (1977). J Exp Med. 145(1): 204–220. doi: 10.1084/jem.145.1.204
2. Kleinman HK, et al. (1986). Biochemistry. 25(2): 312–318. doi: 10.1021/bi00350a005
3. Vukicevic S, et al. (1992). Exp Cell Res. 202(1): 1–8. doi: 10.1016/0014-4827(92)90397-q
4. Hughes CS, et al. (2010). Proteomics. 10(9): 1886–1890. doi: 10.1002/pmic.200900758
5. Waxman EA, et al. (2023). Curr Protoc. 3(2): e681. doi: 10.1002/cpz1.681