

Coating Tissue Culture Plates with Matrigel

Matrigel-GFR (growth factor reduced) #354230 (iPSC culture only)

Matrigel #354234 (iN differentiation only)

0.5 mg of Matrigel gets resuspended in 6 mL of **Cold DMEM/F12** media and coats as following:

Culture plate	Growth area (cm ²)	# Wells 0.5 mg of Matrigel will coat (highlighted numbers indicate volume of resuspension)
24-wells plate	2.0	Resuspend aliquot in 12 mL – 24 wells (0.5mL/well)
12-wells plate	4.0	Resuspend aliquot in 6 mL - 12 wells (0.5mL/well)
6-wells plate	9.5	Resuspend aliquot in 6 mL - 6 wells (1mL/well)
10-cm dish	55	Resuspend aliquot in 6 mL - 1 dish (6mL/well)
96-wells plate	0.32	Resuspend aliquot in 10.8 mL - 180 wells (60uL/well)

(Use Matrigel at 8.7 µg/cm²)

1. Aliquot appropriate volume of cold DMEM-F12 from 4°C and grab Matrigel from – 20°C freezer and keep it on ice.
2. Pipet cold DMEM-F12 to resuspend Matrigel and then filter through 40µM cell strainer.
3. Transfer Matrigel media into your culture plates.
4. Incubate at 37 °C for more than 30-45 min (~1hr).
5. Aspirate Matrigel and wash 1X with PBS. Plate cells immediately.

* Matrigel matrix will gel rapidly at 22°C to 35°C .

* If Matrigel coated plates are not used on the same day as coating, add PBS to prevent evaporation and store in 4°C for up to 10 days.

Matrigel Aliquots

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Matrigel arrives in a 10mL of bottle with varying concentration across lots. Check the protein concentration and aliquot into 0.5 mg and 1mg amounts. Larger amounts can be aliquoted depending on the number of plates you want to use at 1 time.

***Use Matrigel at $8.7 \mu\text{g}/\text{cm}^2$

The day before aliquoting place the following in a 4°C fridge:

1. Autoclaved Eppendorf tubes (for small aliquots - 0.5mg); conical tubes can be used for larger amounts of Matrigel (1mg and up)
2. Eppendorf tube racks
3. Pipette tips
4. Thaw out Matrigel by submerging the bottle in ice and storing in the 4°C overnight.

Aliquoting (once thawed):

- a) Using the cold tips, aliquot the appropriate volume of Matrigel into the cold Eppendorf tube or conical tube.
- b) Keep aliquots on ice!**
- c) Change to a new cold pipette tip after a few tubes to keep it cold
- d) Label the tubes and storage box and store the aliquots in -20 °C

Alternative coating substrate for iN d4 cells

Coating tissue culture plates with poly-orinthine/Laminin (POL) - Do not use for pluripotent iPSC cells or NGRs

Final coating condition: poly-ornithine (10 $\mu\text{g}/\text{ml}$)/laminin (5 $\mu\text{g}/\text{ml}$)

- Dilute poly-ornithine (1000x dilution from stock of 10 mg/ml) and laminin (200x dilution from stock of 1 mg/ml) into PBS
- Aliquot POL solution into culture plates and incubate at 37°C for 3-24 hr
- POL Coated plates (wrapped with paraffin) can be stored at 4°C for several months