Jan 20, 2022

Matrigel coating cell culture plates

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Matrigel is used to pre-coat cell culture dishes and provide an attachment surface for feeder-free human iPS cells to replicate on.

We use Matrigel from Corning (product number) that is provided in a glass bottle as a concentrated liquid (5ml) with lot-specific dilution information. You can look up the Dilution Factor here:

In the JAX Cellular Engineering lab, we use this lot-specific dilution factor to calculate the volume of cold DMEM/F12 to add to the concentrated 5ml Matrigel so that the final concentration is at 40X. The 40X stock solution is aliquoted to 1ml cryovials (0.5ml volume) and stored in a dedicated box in the -20°C freezer. Individual aliquots are thawed and used to further dilute to 1X directly in a cell culture dish prior to plating iPS cells.

Within our CRISPR editing/single cell cloning pipeline, we most often use Matrigel coated plates in the following 2 protocols:
1. Coating wells of a 24W plate to grow small volumes of iPS cells thawed from Matrix plate minivials
2. Coating wells of a 96W plate to grow picked iPS cell colonies during the clone isolation stage of single cell cloning

JUSTIN.MCDONOUGH 2022. Matrigel coating cell culture plates. **protocols.io** https://protocols.io/view/matrigel-coating-cell-culture-plates-b34dqqs6

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- Matrigel will solidify if warmed up. Work with Matrigel stock while it is cold and only dilute into cold serum-free media (ex. DMEM/F12)
- Matrigel 40X stock is stored in 1ml cryovials in the -20°C. Each cryovial contains 500ul. Once thawed, you can store the stock cryovial at 4°C and use it as needed.
- Do not let Matrigel-coated plates dry out
- Matrigel coated plates can be wrapped in parafilm and stored at 4°C for a few days as long as the excess liquid is not removed from the well. Warm these up to 37°C before aspirating liquid and adding cells.

24W or 96W flat bottom cell culture-treated plate
5-ml or 15-ml conical tube for mixing 1X Matrigel prior to distribution
Sterile 25ml reservoirs
COLD DMEM/F12 or other serum-free media
COLD 40X Matrigel stock cryovials

To avoid warming up Matrigel stock, work deliberately and ensure that all the plastic materials you will need are in the cell culture hood.

1 **24W plate:** In an appropriate-sized tube, prepare enough 1X Matrigel to distribute 300-500ul of solution to each well of the 24W plate as needed.

1.1 Working quickly, dilute cold 40X Matrigel 1:40 in cold DMEM/F12. This works out to 25ul of 40X Matrigel for every 1ml of DMEM/F12

1.2 Mix by pipetting and distribute 300-500ul to each well using the same pipet

1.3 Tap the plate if necessary to ensure the liquid has covered the entire surface of the well. Incubate the plate at 37°C/5%CO_2 for at least 2h.

1.4 After incubation, aspirate the excess liquid. NOTE - the liquid will not bead up like Synthemax does. Have your cells or media on hand to add to the wells to prevent the Matrigel from drying out.

2 **96W plate:** Prepare 1X Matrigel directly in a sterile reservoir with the intention of distributing 50ul per well using an 8- or 12-channel multichannel pipet.

2.1 Working quickly, dilute cold 40X Matrigel 1:40 in cold DMEM/F12 directly in a reservoir in the hood. For each 96W plate you plan to coat, add 75ul of 40X

Matrigel to 3ml of DMEM/F12

2.2 Mix directly in the reservoir with a 5ml pipet. Distribute 50ul to each well using a multichannel pipet.

2.3 Tap the plate if necessary to ensure the liquid has covered the entire surface of each well. Incubate the plate at 37°C/5%CO_2 for at least 2h.

2.4 After incubation, pipet off the excess liquid into a waste reservoir using a multichannel pipet. Have your cells or media on hand to add to the wells to prevent the Matrigel from drying out.