Agnete, March 4, 2019

Protocol for coating with Matrigel

Matrigel, growth factor reduced
Basement membrane protein extract from the EHS mouse sarcoma
Corning ref#354230, 10ml vial, Store at -20°C

Preparing aliquots for coating:

- Thaw Matrigel on ice overnight in the fridge – it's important that the Matrigel is kept ice cold all the time to avoid gelation.
- Also put in fridge to cool: 2 x 50 ml tubes with 47.5 ml DMEM/F12 (31330), **labelled** 15ml tubes ("MG 1:20" +date/batch on side), pipettes and blue tips.
- After thawing, mix 2.5 ml Matrigel into each of the 2 x 50 ml tubes with 47.5 ml DMEM/F12. Mix well. Keep liquid and diluted Matrigel, and aliquot tubes on ice at all time
- Aliquot 5ml diluted Matrigel per 15 ml tube, on ice.
- Store at 20°C.

Coating with MatriGel:

Thaw in cold water + ice a 5ml aliquot of MatriGel (already diluted 1:20).

1. Mix 5ml of cold DMEM/F12 into the MatriGel tube (final: 1:40)
2. Coat wells by pipette the diluted MatriGel solution into plates: 0.8 ml per 12w, 2 ml per 6w (volume can vary with batch, test each batch)
3. Incubate at 37°C for 2-4 hours (or seal plates with parafilm and keep in fridge for up to 2-3 weeks for later usage, then incubate at 37°C for 2-4 hours before use)
4. Before seeding cells, wash wells 1x with DMEM/F12 – swirl plate while washing. Take care that coated wells do never dry out.