Matrigel Handling Entered by Piotr Przanowski
Janes Lab Protocols 2/10/2023

I. Preparing and using Matrigel aliquots:

1. Redissolve growth factor reduced (GFR) Matrigel (Corning #356253) stocks by thawing 10-ml vials overnight on a nutator in the cold room
   - *Newer Matrigel lots can be thawed on ice, but older lots partially desiccate and require constant mixing to redissolve thoroughly*
   - *Do not allow the 10-ml vials to sit for more than 16–18 hr at 4ºC*
2. Place the thawed Matrigel on ice and pipet up and down extensively with a 5 ml stripette to remove any visible or mechanical nonuniformities
   - *Even with overnight nutation, ECM aggregates remain in the vial and can be seen as phenol red-concentrated or overly viscous clumps at the bottom of the vial—there should be <u>no residue</u> in the vial if the Matrigel is thoroughly dissolved*
   - *Pipet vigorously but try to minimize bubbles*
   - *If after long pipetting clumps are still present freeze the Matrigel vial in –80ºC, thaw it on ice and continue pipetting until no clumps is present*
3. Prepare 1-ml aliquots in microcentrifuge tubes on ice and refreeze at –20ºC
   - *Be sure to write the Matrigel lot number on the tubes*
4. For use, thaw 1-ml aliquots by placing on ice for 30–60 min until reliquefied
   - *Surrounding the aliquots with ice will take a very long time to thaw*
   - *Be sure to surround the aliquots with ice after thawed and to remix the aliquot with a 1-ml micropipette before use*
   - *Remaining unused Matrigel can be refrozen, and leftover aliquots can be reused after pooling and remixing them thoroughly*

II. Centrifugal coating of chamber slides with Matrigel:

1. Thaw 1-ml aliquots of Matrigel on ice for 30–60 min until reliquefied
   - *Surrounding the aliquots with ice will take a very long time to thaw*
   - *Be sure to surround the aliquots with ice after thawed and to remix the aliquot with a 1-ml micropipette before use*
   - *Remaining unused Matrigel can be refrozen, and leftover aliquots can be reused after pooling and remixing them thoroughly*
2. In a biosafety cabinet, add 30 µl of Matrigel as a drop to the middle of each chamber of two eight-well chamber slides (Fisher #354108)
   - *Use reverse pipetting to avoid bubbles when dispensing the Matrigel*
3. Incubate for 3 min at room temperature and centrifuge for 10 min at 2500 rcf *at 4ºC* on the Sorvall Legend RT+ centrifuge with the swinging bucket rotor using hanging 96-well plate adaptors
   - *The label of the chamber slide should face away from the middle of the centrifuge, with the chamber slide positioned in the middle of the adaptor and as far away from the rotor as possible (see image)*
   - *The centrifugal force of the rotor uniformly spreads out the Matrigel as it solidifies*
   - *2500 rcf is ~3500 rpm on our rotor*
   - *Do not place more than one slide per 96-well plate adaptor*
4. Plate cells withing one hour of chamber slide coating
   - *After ~two hours, the Matrigel will dry out, become overly rigid, and begin cracking*
   - *Do not place the chamber slides in the 37ºC incubator until cells have been plated*


SORVALL
Heraeus
Middle of the centrifuge
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