Matrigel Test

We obtain Growth Factor Reduced Matrigel from BD Biosciences (BD No. 354230). Thaw on ice overnight at 4C. Matrigel remains liquid on ice but solidifies rapidly when warmed, so it should be handled on ice at all times. Once thawed, the Matrigel can be stored as 1.0-ml aliquots at -20C.

Because there is lot-to-lot variability in Growth Factor Reduced Matrigel, the lab should test individual lots, prior to purchasing large quantities for experiments. Contact Andrea L Bramwell <BramwellAL@corning.com> to request protein concentration and endotoxin of multiple lots. In determining the appropriateness of a lot for 3D assays, we prefer those with protein concentrations ranging from 10 to 12 mg/ml and an endotoxin level less than 2 units/ml. For any interesting experimental observation, it is advisable to repeat the assay with two or more independent lots of Matrigel to confirm the generalizability of the result. Pick 3 lots with reasonable protein concentration and endotoxin level for further morphology test.

To test the level of residue growth factor, we used the morphology of MCF-10A cells in Matrigel as readout. Desirable Matrigel lot with minimum growth factor should yield single acini with well-polarized outer layer of cells, intact basement membrane with no invasive phenotype. By day 10+ of culture, the center of acini should form a hollow lumen from cell apoptosis. (As shown below)

Three-Dimensional Morphogenesis of Mammary Epithelial Cells (MCF-10A):

1. Thaw Matrigel on ice (usually takes a long time and will depend on the size of the aliquot). Note: Matrigel will remain as liquid on ice but will solidify at room temperature.

2. Add 40ml of Matrigel to each well of 8-well glass chamber slide (@ 50ml/cm2) (Falcon # 354108) and spread the Matrigel evenly in the well using a yellow tip. Spread the Matrigel gently, if you try to overdo the spreading, Matrigel will form a high meniscus at the edges. Take care not to generate air bubbles. You may want to do this step on ice until you get used to the procedure.

3. Place the slides in the cell culture incubator and allow the Matrigel to solidify (takes 15-20 min).

4. Trypsinize cells and re-suspend in 1.0ml of media containing 20% FBS (no other additives). Add additional 2.0 ml of 20% FBS containing media and incubate the cells for 1-2 minutes at RT (this step ensures inactivation of trypsin).

5. Spin the cells at 900rpm for 3 minutes.

6. Re-suspend the cells in 10 ml of Assay medium.
7. Count cells and aliquot 100,000 cells in to a fresh 15 ml conical tube. Bring up the volume to 4.0 ml using Assay media (Label this tube as "A"). Final concentration will be 2,500 cells/100ml.

8. Prepare Assay media containing 5% Matrigel and 10ng/ml EGF (Label this tube "B"). Use media that is cooled on ice – doesn't have to be ice cold! Since the EGF stock solution is stored at 100mg/ml, it is advisable to prepare a working dilution of 10mg/ml using Assay media. This diluted stock should not be stored for extended use, EGF stock solutions less than 100 mg/ml will not remain stable. Attempting to aliquot very small volumes from the 100 mg/ml stock will likely be a source of experimental error.

9. Mix Tube A and Tube B 1:1 and plate 400 ml of the Matrigel:Cell mix onto the solidified Matrigel (Step 3).

10. Gently transfer the chamber slide into the incubator.

11. Change media once every 4 days with Assay media containing 2.5% Matrigel and 5ng/ml EGF. MCF-10A cells take about 8-12 days to form acini-like structures. You may choose to record images every 2-3 days.

	Growth Media	**Assay Media**	**Resusp. Media**
DMEM/F12	500.00ml	500.00ml	400.00ml
Horse Serum	25.0 ml	10.0 ml	100.00ml
EGF (100micro.g/ml)	100µl	--	--
Hydrocortizone (1mg/ml)	250µl	250µl	--
CholeraToxin (1mg/ml)	50µl	50µl	50 µl
Insulin (10mg/ml)	500µl	500µl	--
Pen/Strep	5.0ml	5.0ml	5.0ml

Note: Premix all the all the additives (Serum, EGF, etc.,) filter through a 0.2µm filter and then add it to DMEM/F12 media.

'Growth media' can be stored for a month at 4ºC.

DMEM/F12: GIBCO-BRL

EGF: R&D Systems; Cat# 236-EG

Hydrocortizone: Sigmal; Cat# H-0888. Make stock soln in 95% ethanol and store at –20C.

CholeraToxin: Sigma; Cat# C-8052. Make stock soln in water and store at 4C.