Three Dimensional Growth of MCF10A in Matrigel

1. Trypsinize a confluent plate of cells and resuspend in 1 ml DMEM/F12 + 20% horse serum. Add an additional 3 mls to the tube to fully quench trypsin.

2. Add 45 μl of Matrigel to each well of an 8-well chamber slide (BD Falcon *CultureSlide*) and spread evenly in the well. Take care not to generate air bubbles or touch the pipette tip to the edge of the well (this will form a high meniscus on the border). Place the slides in a cell culture incubator to allow the Matrigel to gel completely (takes 30-40 min).

3. Spin the cells at 900 rpm for 3 min.

4. Resuspend the cells in a total of 10 mls Assay Media (same as 10A growth media, but with 2% serum and no EGF).

5. Count cells using a hemacytometer. Calculate the number of cells needed for each cell line (5000 cells/well) and the corresponding volume from the 10ml cell suspension.

6. Prepare a stock solution of Assay Media with 5ng/ml EGF and 2% Matrigel for the total number of wells + 1 (400 μl overlay media / well).

7. Transfer an appropriate amount of overlay media (n + 1 wells/cell line x 400 μl) to a fresh tube and add the respective volume of cell suspension.

8. Plate 400 μl of above mixture to a well on top of the solidified Matrigel. This should give you 5000 cells/well in media containing 2% Matrigel and 5 ng/ml EGF or other stimulant.

9. Allow the cells to grow in the incubator for approximately 10-15 days. The cells should be refed with fresh Assay Media, 2% Matrigel and 5 ng/ml EGF (or other stimulant) every four days. (Think of the day you set up the assay as day 0, then feed on days 4, 8, 12, 16, etc.). The cells should form spheres by about day 5-6 and then start forming hollow lumen by day 7-8.

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