Overlay Three-Dimensional Culture of MCF-10A Cells on Matrigel™

1) Handling Matrigel™: We obtain Growth Factor Reduced Matrigel™ from BD Biosciences (BD#354230). Thaw on ice overnight at 4°C. Matrigel™ will stay as liquid on ice but will solidify 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 –20°C. Because there is lot-to-lot variability in Growth Factor Reduced Matrigel™, our laboratory tests individual lots, prior to purchasing large quantities for experiments. In determining the appropriateness of a lot for 3-D assays, we prefer those with protein concentrations ranging from 10-12 mg/ml and an endotoxin level of 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.
2) Add 45 μl of Growth Factor Reduced Matrigel™ to each well of an 8-well glass chamber slide and spread evenly in the well using the tip of a P-200 pipetman. Take care not to generate air bubbles or overspread; this will form a high meniscus on the border. The chamber slide can be pre-cooled on a tray of ice, which provides extra time to spread the EHS. Place the slides in a cell culture incubator to allow the basement membrane to solidify for at least 15 minutes. The coated chamber slides must be placed promptly in the cell culture incubator to avoid dehydration.
3) While the Matrigel™ is solidifying, trypsinize a confluent plate of cells (See Protocol 1 for details) and resuspend in 3-5 mls Resuspension Medium, transfer to 15 mls conical tube.
4) Spin the cells at 150xg in a tissue culture centrifuge for 3 min.
5) Resuspend the cell pellet in 2 ml of "Assay Medium" (See Media Table for recipe) lacking EGF. Resuspend cells 10 to 25 times with a P1000 pipetman to ensure that a single cell suspension has been obtained. Then, add additional Assay Medium to achieve a final volume of 8 to 10 ml.
6) Count cells.
7) Prepare a stock of Assay Medium with 2% Matrigel™ and 5ng/ml EGF – it is necessary to make 400 μl of this stock for each well of the chamber slide; furthermore it is recommended that enough stock solution for "n+1" assays be prepared to account for volumetric errors in pipetting.
8) Add the desired number of cells (6,000 cells/well – in general in a small volume to avoid significant dilution) to the appropriate amount of stock medium from step 7).
9) Plate 400 μl of this mixture on top of the solidified Matrigel™ in each well of the chamber slide. This corresponds to a final overlay solution of 6,000 cells/well in medium containing 2% Matrigel™ and 5 ng/ml EGF.
10) Allow the cells to grow in a 5% CO_2 humidified incubator at 37°C. The cells should be refed with Assay Medium containing 2% Matrigel™ and 5 ng/ml EGF every four days. (The day that the assay is set up corresponds to day 0; thus, feed on days 4, 8, 12, 16, etc.). The cells should form clusters by day 5-6 of 3-D cultures and subsequently start forming hollow lumen.