MiCA: 3D Culture of MCF10A Breast Epithelial Cells

v.01

Description: Growth of MCF10A cells in three dimensional laminin-rich extracellular matrix (3D lr-ECM) is a well studied model for human breast epithelial acinus formation (1). Notably, many key biological properties relevant to breast cancer research are promoted in 3D culture as compared with 2D culture (2). This protocol describes using the MiCA plate for long term perfusion culture of MCF10A cells in lr-ECM.

Preparing Cells:

1. MCF10A cells (ATCC #CRL-10317) are maintained in 2D culture with the recommended medium (DMEM/F12 supplemented with 5 ng/ml EGF, 2% horse serum, 0.5 µg/ml hydrocortisone, 10 µg/ml insulin, 100 ng/ml Cholera toxin). When ready for use, cells are trypsinized (0.05% trypsin in 1X PBS) for 15 minutes, neutralized with soybean trypsin inhibitor (0.1% trypsin inhibitor in 1X PBS), and resuspended in culture medium at 1,000-4,000 cells/µl.
2. Thaw lr-ECM (Matrigel, BD Biosciences #354234) undiluted at 4°C to attain liquid state.

Loading 3D Embedded:

3. Mix Matrigel with cell suspension at a ratio of 8:1 (gel:cells) using a pipette, being careful not to introduce air bubbles. The gel:cell ratio can be varied based on the desired matrix properties. Store on ice to prevent gel polymerization.
4. Empty the wells of the MiCA plate as instructed in the "MiCA Product Sheet." Pipette 4 µl of cell/gel mixture into each open top culture chamber.
5. Place in a 37°C incubator for 10-30 minutes to polymerize gel.

Loading 3D Overlay (Sandwich):

6. Empty the wells of the MiCA plate as instructed in the "MiCA Product Sheet." Pipette 1 µl of Matrigel into the bottom of the open top culture chamber. Place in a 37°C incubator for 10-30 minutes to polymerize gel.
7. Add 3 µl of the cell suspension to the open top culture chamber. Wait 5 minutes for the cells to settle to the bottom.
8. Add 3 µl of Matrigel to the open top culture chamber. It is not necessary to remove the excess medium from step 7. Place in a 37°C incubator for 10-30 minutes to polymerize gel.

Perfusion Culture:

9. Pipette 300 µl of culture medium to the flow inlet well to initiate gravity driven flow. The medium will perfuse at a rate of ~100 µl/day.
10. For long term culture , refill the inlet well and empty the outlet well every 2-3 days.

References:

1. *Lee, et al. Nat Methods, 2007. 4(4):359-65.*
2. *Kenny, et al. Mol Oncol, 2007. 1(1): 84-96.*

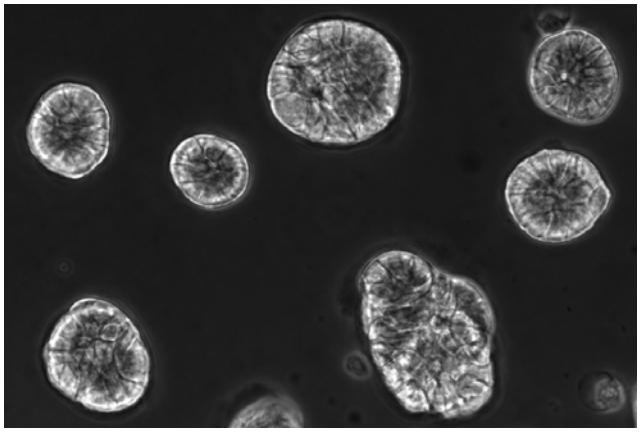

Figure 1. 3D acinar structure of MCF10A cells in the MiCA plate.

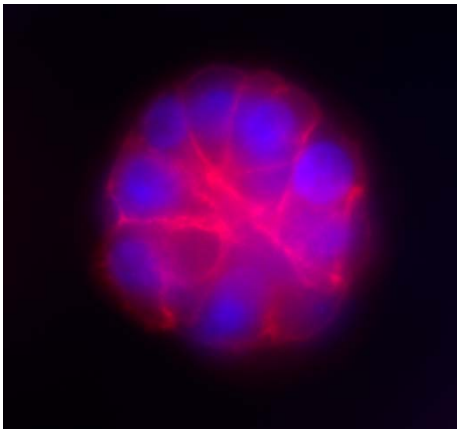

Figure 2. Actin (red) and DNA (blue) stain of 3D acinus.