OkaSciences

In Vitro 3D Embedded Cell Culture - 24 Well Plate

Overview:

OkaMatrix is a basement membrane matrix material composed of laminin, collagen IV, enactin and heparan sulfate proteoglycans to accurately mimic the *in vivo* extracellular matrix environment. OkaMatrix is free from undefined growth factors and enzymes.

Protocol:

Preparation Notes:

- All equipment coming in contact with OkaMatrix must be pre-chilled/ice cold to ensure it remains a liquid while in use. OkaMatrix will irreversibly solidify at 37°C.
- OkaMatrix will begin to gel at 10°C.

1. Thaw OkaMatrix by placing on ice and in a 4°C refrigerator overnight. Once thawed, swirl the vial to ensure OkaMatrix is evenly distributed.
2. Dilute the OkaMatrix to 5mg/ml (from 8.18mg/ml starting concentration) with ice-cold complete cell culture media.
3. Add 100ul of diluted OkaMatrix into each well of the 24 well plate. Spread OkaMatrix evenly across the well using the pre-chilled pipette tip.
4. Incubate the plate at 37°C for 30 minutes to allow OkaMatrix to gel.
5. Prepare cell pellet.
    a. Only use healthy cells that are less than 75% confluent.
    b. Wash cells once with PBS, then make a single-cell suspension using your preferred cell dissociation method.
    c. Centrifuge cells at 125 x g for 5 minutes at room temperature.
6. Resuspend the cells with complete cell culture media to achieve a final cell density of 5 x 10^6 cells/ml.
7. Add 30ul of the prepared cell suspension to 270ul of diluted OkaMatrix solution to achieve final cell density of 5 x 10^5 cells/ml.
    a. The volume of the cells should not exceed 10% of the OkaMatrix solution to ensure OkaMatrix can properly polymerize into the matrix. The final number of cells may need to be optimized based on the growth properties of the cell line.
8. Incubate plate at 37°C for 30 to 45 minutes.
9. Gently add 500ul of complete cell media to each well.
10. Continuously culture cells for 8 to 10 days by changing complete cell culture media every 2 days.