Layered OkaMatrix 3D Cell Culture Protocol - 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. Add 200ul of OkaMatrix to each well of a pre-chilled 24 well plate. Spread OkaMatrix evenly across the well using a pipette tip.
3. Incubate the plate at 37°C for 30 minutes to allow OkaMatrix to gel.
4. Prepare cell pellet.
    - Only use healthy cells that are less than 75% confluent.
    - Wash cells once with PBS, then make a single-cell suspension using your preferred cell dissociation method.
    - Centrifuge cells at 125 x g for 5 minutes at room temperature.
5. Resuspend cells with complete cell media to achieve a final cell density of 3 x 10_5 cells/mL. Note the concentration of cells may need to be adjusted based on the growth properties of the particular cell line used.
6. Plate 250ul of the cell suspension into each well of the newly coated plate.
7. Incubate at 37°C for 30 minutes.
8. Create OkaMatrix medium mixture
    - Chill cell culture media on ice for minimum 20 minutes to ensure complete cooling.
    - Add OkaMatrix to 10% of the final volume of culture media
    - Pipette mixture up and down several times to ensure complete mixing.
9. Gently add 250ul of the OkaMatrix medium mixture to the plated culture.
10. Continuously culture cells for 4 to 7 days by changing OkaMatrix medium mixture every 2 days.