Fennik Life Sciences

3D- Matrigel-Collagen assay protocol in 96 well plates

1. Thaw Matrigel on ice.
2. Prepare rat tail collagen type 1 (working concentration 2.5 mg/ml-4.0 mg/ml) by adjusting pH to neutral with prepared lab setting solution that has phenol red. Start by mixing rat tail collagen and setting solution in a 4:1 ratio. Adjust as necessary until the mixture turns to a light pink/orange color by adding 2-5ul of either setting solution or rat tail collagen. For recipe to setting solution, see below. Leave collagen on ice, until you are ready to use.
3. Mix rat tail collagen (prepared above) with thawed reduced factor Matrigel matrix in a 1:1 ratio (specific volumes depend on the number of wells to be coated therefore calculate and determine required volumes prior to your experiment).
4. While maintaining the 96 well plate on ice, coat each well of the plate that you plan to use for your assay with 40ul of the Matrigel/collagen mix prepared above. Use a 200ul pipette to ensure that there is a uniform distribution of the Matrigel/collagen mix on the bottom of the well. Avoid bubbles; you may centrifuge the plate to ensure a homogenous distribution of the Matrigel/collagen mix. Allow the Matrigel/collagen mix to solidify by placing plate in cell culture incubator for 30-60 minutes.
5. While the Matrigel/collagen is solidifying; prepare the **assay medium,** which is 5% Matrigel in tissue culture medium (for example; add Matrigel to DMEM/10% FBS).
6. Determine how many cells you want to plate per well. Recommended starting cell number= 2500/well. Trypsinize cells. Count cells and resuspend cells at 2500 cells/100ul in regular cell culture medium for that cell line. Depending on number of wells you intend to plate, it is recommended that users prepare a master mix of cells to minimize possible variations in cell number/well.
7. Mix cells with the previously made **assay medium** in a 1:1 ratio : 100 ul of cells: 100 ul **assay medium**. Pipette up and down several times to mix thoroughly.
8. Add 200ul of cell/assay medium mix to the coated wells. Gently shake plate to get uniform distribution of cells.
9. Leave the plate in the cell culture incubator at 37^{o}C.
10. Change media every 2 days. Remove conditioned media gently using a 200ul pipette. Add 200ul of fresh **assay medium**.

11. Observe spheroids under the microscope at 10X and capture the images.

Setting solution recipe:

10xEBSS (Gibco) 100ml
NaHCO3 2.45g
1M NaOH 7.5ml
Sterile Distilled Water 42.5ml

For long-term storage, aliquots can be frozen at -20ºC or -80ºC.

References: protocols adapted from:

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