Fennik Life Sciences

3D- Collagen assay protocol in 96 well plates

1. Determine how many cells you want to plate per well. Users may need to optimize cell number for their cell line and experiment. Recommended ranges: 2500-25,000/well.
2. 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. To do so:
    a. Trypsinize cells. Quench trypsin with serum containing media
    b. Count cells.
    c. Aliquot the total number of cells you need into an Eppendorf or conical tube. Centrifuge cells for 5 min at 0.3 g.
    d. Aspirate or pipet media out. Leave cell pellet
    e. Resuspend cells at desired cell number/100ul rat tail collagen for that cell line.
3. Prepare rat tail collagen type 1 (working concentration range: 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.
4. Add 100 ul collagen mix containing cells into each well of a 96 well plate
5. Gently shake plate to get uniform distribution of cells.
6. Incubate plate in cell culture incubator at 37^{o}C for 15 minutes to allow collagen to polymerize.
7. Add 100-200 ul of media to each well.
8. Change media every 2 days. Remove old media gently using a 200ul pipette.
9. Observe cells under the microscope 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: adapted from:

Hayward, S.W. et al. Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model Differentiation 1998 Jul;63(3):131-40. doi: 10.1046/j.1432-0436.1998.6330131.x.

Fang et al. Targeted gene silencing of CCL2 inhibits triple negative breast cancer progression by blocking cancer stem cell renewal and M2 macrophage recruitment. Oncotarget. 2016 Jun 7. doi: 10.18632/oncotarget.9885. [Epub ahead of print] PubMed PMID: 27283985