Fennik
Life Sciences

Establishing 3D collagen cultures in TheraKan™ devices

1) To embed cells in collagen, it is necessary to lower the pH of the collagen to achieve polymerization by mixing the collagen with a setting solution. Prepare the setting solution by mixing 100 ml of 10X EBSS with 2.45 g of $NaHCO_3$, 7.5 ml of 1 M NAOH and 42.5 ml of sterile distilled water. Further sterilize using a bottle top 0.22 micron filter unit attached to a sterile bottle.

2) Mix the rat tail collagen I (recommended starting stock concentration: 2.5 mg/ml) with setting solution at a starting 4: 1 ratio. For 1 device, use 275-300 μl of collagen with 50-75 μl of setting solution in an eppendorf tube. Vortex briefly or pipet the sample up and down to mix the sample thoroughly. Note, volumes can be adjusted as desired.

3) Add collagen or setting solution at 5-10 μl increments and mix thoroughly until the phenol red dye in the mixture changes to a light pink to orange color, reflecting a neutral pH. A yellow color indicates acidic (low) pH while dark pink reflects basic or high pH. Keep the mixture, cold on ice as higher temperatures will accelerate polymerization.

4) Trypsinize and count cells. Mix cells with collagen: setting solution, recommended starting cell number/device: 100,000 cells/250 μl.

5) Swivel TheraKan™ devices closed. Add collagen:cell mixture to device. Place devices in test chambers. Incubate collagen gels at 37°C for 10-20 minutes to polymerize.

6) Add 1-2 ml of media to device. Recommend incubating 3D cultures for 24 hours before starting assays.

7) To examine flow of molecules or cells in/out of devices, open devices. Pipet media/cells into test chamber, and assay for desired length of time.