

***In vitro* Angiogenesis Assay: Endothelial Cell Tubule Formation in Matrigel**

1. Thaw matrigel overnight at 4°C on ice
2. Cool microplate and pipette tips at -20°C
3. Add to 150µl Matrigel per well on a 48-well microplate
4. Incubate for 30 minutes in 37°C incubator
5. Trypsinize and neutralize cells
6. Rinse with 5ml HEPES
7. Pellet cells at 200g for 5 minutes, aspirate supernatant
8. Resuspend in 1mL of EBM.
9. Determine cell concentration by hemocytometry
10. Dilute to a final concentration of 600 cells/µL
11. Plate 30,000 cells per well (50 µL of 600 cells/µL suspension)
12. Add 50µL patient/control plasma
13. Capture two 4X images at 12 hours