Protocol for coating 100 ng/mL VEGF bound plate:

1. Prepare the following solution in 1X PBS:
   - The stock solution for
     - Fibronectin: **1 mg/mL**
     - VEGF: **1 µg/µL**
   - Working solution:
     - **12.5µg/mL** FN
     - **0.5µg/mL** VEGF
2. Adjust the volume accordingly. The volume below is used for 10cm plate (5 ml).
   - For Bound VEGF plate, add 4 ml of FN in working solution on the plate, then add 1 ml of VEGF in working solution.
     - This gives you VEGF concentration at 100ng/mL. The total VEGF amount on the plate will be around 0.5µg.
   - For soluble VEGF plate, add 4 ml of FN in working solution, then add 1ml of 1X PBS.
3. Incubate at 37°C for 4 hour. Shaking to make sure the molecule disperse evenly.
4. After 4 hours, wash 2 or 3 times with 1X PBS or serum-free media.
5. Tripsinize cells,
   - For bound plate:
     - Put down the cells directly.
   - For Soluble plate:
     - Resuspend the cells into 4 ml of medium.
     - Add 1 ml of VEGF in working solution to make final concentration 100ng/mL.
     - Add the cells mixed with VEGF on the plate right away.