

Protocol: Coating of Transwell Filters

- I) **Fibronectin** (Human Fibronectin from BD Biosciences (Cat# 354008) 1mg lyophilized)
- A) Stock Solution
 - 1) Dissolve in 1ml MilliQ H₂O to **1 mg/ml**
 - 2) Aliquot into 30 ul aliquots and freeze at -20C
 - B) Dilute stock solution 1:20 in MilliQ H₂O to **50 ug/ml**
 - 1) For 12 – 6.5mm Transwell filters: 30ul stock solution in 600ul MilliQ H₂O
 - C) Add 50 ul to apical chamber of each 6.5mm well (**7.5 ug/cm²**)
 - D) Incubate for 2 hours at 37C
 - E) Aspirate remaining solution out of apical chamber
 - F) Let dry for 30 minutes under tissue hood
 - 1) Filters can be wrapped in parafilm and stored at 4C for up to two weeks
 - G) Add media to apical and basolateral compartments and incubate at 37C for 30 minutes prior to cell seeding
- II) **Collagen I** (Rat tail Collagen I from BD Biosciences (Cat# 354236) 100 mg in 0.02N acetic acid (3.85 mg/ml))
- A) Stock Solution (**3.85 mg/ml**) stored at 4C
 - B) Dilute stock solution to **50 ug/ml**
 - 1) For 12 – 6.5mm Transwell filters: 7.8ul stock solution in 600ul MilliQ H₂O
 - 2) For 6 – 24.5mm Transwell filters: 55ul stock solution in 4200ul MilliQ H₂O
 - 3) For 10cm dish: 39ul stock solution in 3000ul MilliQ H₂O
 - 4) For T25 dish: 19.5ul stock solution in 1500ul MilliQ H₂O
 - C) Add diluted solution to filter or dish
 - 1) For 6.5mm Transwell filters: add 50ul to apical chamber of each well (**7.5 ug/cm²**)
 - 2) For 24mm Transwell filters: add 700ul to apical chamber of each well (**7.5 ug/cm²**)
 - 3) For 10cm dish: add 3ml (**2.73 ug/cm²**)
 - 4) For T-25 flask: add 1.5ml (**3 ug/cm²**)
 - D) Incubate for 2 hours at 37C
 - E) Aspirate remaining solution out of apical chamber
 - F) Let dry for 30 minutes under tissue hood
 - 1) Filters can be wrapped in parafilm and stored at 4C for up to two weeks
 - G) Add media to apical and basolateral compartments and incubate at 37C for 30 minutes prior to cell seeding
- III) **Collagen IV** (Human Collagen IV from BD Biosciences (Cat# 354245) 0.25mg in 0.167ml 10mM acetic acid (1.5mg/ml))
- A) Stock Solution (**1.5 mg/ml**)
 - 1) Aliquot to 5 & 10 ul and store at -80C
 - B) Dilute stock solution 1:30 in MilliQ H₂O to **50 ug/ml**
 - 1) For 12 – 6.5mm Transwell filters: 20 ul stock solution in 600ul MilliQ H₂O
 - C) Add 50ul to apical chamber of each well (**7.5 ug/cm²**)
 - D) Incubate for 2 hours at 37C
 - E) Aspirate remaining solution out of apical chamber
 - F) Let dry for 30 minutes under tissue hood
 - 1) Filters can be wrapped in parafilm and stored at 4C for up to two weeks
 - G) Add media to apical and basolateral compartments and incubate at 37C for 30 minutes prior to cell seeding