IncuCyte™ ClearView™ Plate Coating Protocols

The IncuCyte™ Chemotaxis Cell Migration Assay utilizes a low pore density membrane; requiring cells to migrate across the membrane surface in order to reach a pore. Cell-surface interactions are critical to the migration process and we have found that many cell types require an extracellular matrix (ECM) to promote cell attachment and migration. Outlined below are recommended coating procedures for commonly used extracellular matrices. These protocols are not optimized for every cell type. Optimization of ECM concentration as well as the requirement of top-side only coating versus coating both sides of the membrane, may need to be evaluated in order to achieve optimal assay performance.

NOTE: To reduce bubbles a reverse pipetting technique should be used for all liquid transfers into reservoirs and inserts.

How to Video **Coating an IncuCyte™ ClearView 96-Well Cell Migration Plate**
https://www.youtube.com/watch?v=YEOPmxsp-oI

Coating with Protein-G/ICAM-1

Materials

- Protein G (Life Technologies 101200)
- ICAM (Life Technologies 10346-H03H)
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)
- Bovine Serum Albumin (BSA; Sigma Aldrich, A7906)

1) Coat the top surface of the IncuCyte™ ClearView™ Plate membrane with 20 µL of 20 µg/mL Protein G solution for 1 hour at 37 °C
2) Wash the membrane once with D-PBS by adding 40 µL D-PBS directly to the wells containing Protein G. Remove the full volume (~ 60 µL) and promptly proceed with the ICAM coating step.
3) Coat the top of the membrane with 20 µL of 5 µg/mL ICAM for 2 hours at 37 °C.
4) Block both sides of the membrane with D-PBS + 1% BSA by adding 20 µL to the insert wells and 150 µL to the reservoir wells (pre-fill reservoir and gently place the insert into the reservoir plate containing BSA). Incubate at ambient temperature for 30 minutes.
5) After incubation, transfer the insert plate to a new reservoir containing 200 µL of PBS in each well. Immediately prior to cell addition, wash the insert wells once with D-PBS as described in step 2 above.

Coating with Matrigel®

Materials

- Matrigel® (Corning 354234, follow manufacturer's instructions for keeping stock Matrigel® cold at all times)
- Fetal Bovine Serum (Sigma-Aldrich F2442) – *optional, depending on cell type*
- Chemotaxis assay medium – *cell type dependent*
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)

1) Coat both sides of the IncuCyte™ ClearView™ Plate membrane with 50 µg/mL Matrigel® diluted in chemotaxis assay media (optional, + 10% FBS) by adding 20 µL to the insert wells (reverse pipette) and 150 µL to the reservoir wells (pre-fill reservoir and gently place the insert into the reservoir plate containing coating matrix). In this case, a second reservoir plate will be loaded with chemoattractant and used during the experiment.
   NOTE: The chemotaxis plate must be pre-chilled to 4 °C. We recommend using a CoolSink™ to keep the plate cold during the coating procedure.
2) Place the plate at 37 °C and incubate for 30 minutes.
3) Remove the plate from 37 °C and allow to cool down to ambient temperature for 30 minutes.
   NOTE: This step is important in order to achieve even cell distribution.
4) Aspirate the Matrigel® coating from the reservoir wells. Add 200 µL of D-PBS to the reservoir and gently return the insert into to the reservoir plate.
5) Prior to cell seeding, aspirate the Matrigel® coating from the insert.
   NOTE: No washing of the insert is required prior to cell seeding.

Coating with Fibronectin

Materials

- Fibronectin (Sigma Aldrich, F1141)
- Bovine Serum Albumin (BSA; Sigma Aldrich, A7906) – *optional, used to reduce bubble formation*
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)

1) Prepare fibronectin at concentrations suitable for the cell type being tested. We recommend testing concentrations of 5 - 50 µg/ml in D-PBS (without calcium or magnesium) supplemented with 0.1% BSA.
2) Pipette 150 µl of fibronectin solution into the IncuCyte™ ClearView™ reservoir plate. Place the IncuCyte™ ClearView™ Plate insert into the reservoir and pipette 20 µl of the fibronectin solution into the insert. In this case, a second reservoir plate will be loaded with chemoattractant and used during the experiment.
3) Incubate for 1 hour at ambient temperature.
4) Aspirate the fibronectin + 0.1% BSA coating from the reservoir wells and replace with 200 µL of D-PBS and gently return the insert into to the reservoir plate.
5) To the insert, add 40 µL of D-PBS to the wells containing fibronectin + 0.1% BSA, then aspirate the full volume (~60 µL) prior to cell seeding.

Coating with Collagen

Materials

- Collagen Rat Tail Type I (BD 354236) or Collagen Rat Tail Type IV (BD 354233)
- 0.02N Acetic acid – *for dilution of Collagen Type I*
- 0.05M HCl - *for dilution of Collagen Type IV*
- Bovine Serum Albumin (BSA; Sigma Aldrich, A7906) – *optional, used to reduce bubble formation*
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)

1) Prepare collagen at concentrations suitable for the cell type being tested. We recommend a concentration of 50 µg/ml of Collagen Type I diluted in 0.02N acetic acid supplemented with 0.1% BSA, or if using Collagen Type IV, we recommend a concentration of 5 µg/mL diluted in 0.05M HCl.
2) Pipette 150 µl of collagen solution into the IncuCyte™ ClearView™ reservoir plate. Place the IncuCyte™ ClearView™ Plate insert into the reservoir and pipette 20 µl of the collagen solution into the insert. In this case, a second reservoir plate will be loaded with chemoattractant and used during the experiment.
   NOTE: To reduce bubbles a reverse pipetting technique should be used for all liquid transfers into reservoirs and inserts.
3) Incubate for 1 hour at ambient temperature.
4) Aspirate the collagen coating from the reservoir wells and replace with 200 µL of D-PBS and gently return the insert into to the reservoir plate.
5) To the insert, add 40 µL of D-PBS to the wells containing collagen, then aspirate the full volume (~60 µL) prior to cell seeding.

Coating with Gelatin

Materials

- Attachment Factor Protein (AF), containing 0.1% gelatin (Life Technologies S-006-100)
- Fetal Bovine Serum (Sigma-Aldrich F2442) – *optional, depending on cell type*
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)

1) Pipette 150 µl of 1x AF solution into the reservoir. Place the IncuCyte™ ClearView™ Plate insert into the reservoir and pipette 20 µl of the AF solution into the insert. In this case, a second reservoir plate will be loaded with chemoattractant and used during the experiment.
2) Incubate for 2 hours at ambient temperature or for 30 minutes at 37 °C.
   NOTE: If incubating at 37 °C, we recommend cooling the plate at ambient temperature for 15 – 30 minutes prior to cell seeding.
3) Prior to cell seeding, aspirate AF solution from the insert well and reservoir wells. To the reservoir, aliquot 200 µL of D-PBS and gently return the insert into to the reservoir plate.
   NOTE: No washing of the insert is required prior to cell seeding.

Coating with Laminin

Materials

- Laminin (Life Technologies 23017-015, follow manufacturer's instructions for keeping stock laminin cold at all times)
- Fetal Bovine Serum (Sigma-Aldrich F2442) – optional, depending on cell type
- Chemotaxis assay medium – cell type dependent
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)

1) Coat both sides of the IncuCyte™ ClearView™ Plate membrane with 25 µg/mL Laminin diluted in chemotaxis assay media (optional, + 10% FBS) by adding 20 µL to the insert wells (reverse pipette) and 150 µL to the reservoir wells (pre-fill reservoir and gently place the insert into the reservoir plate containing coating matrix). In this case, a second reservoir plate will be loaded with chemoattractant and used during the experiment.
   NOTE: The chemotaxis plate must be pre-chilled to 4 °C. We recommend using a CoolSink™ to keep the plate cold during the coating procedure.
2) Place the plate at 37 °C and incubate for 30 minutes.
3) Remove the plate from 37 °C and allow to cool down to ambient temperature for 30 minutes.
   NOTE: This step is important in order to achieve even cell distribution.
4) Aspirate laminin coating from the reservoir wells and replace with 200 µL of D-PBS and gently return the insert into to the reservoir plate.
5) To the insert, add 40 µL of D-PBS to the wells containing laminin, then aspirate the full volume (~60 µL) prior to cell seeding.