Matrigel Coating and Cell Invasion Protocol Using the CIM-Plate 16
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This protocol is designed and optimized for detection of cell invasion with the CIM-Plate 16 and the RTCA DP Instrument using Matrigel basement membrane matrix. Matrigel is a meshwork of ECM proteins and growth factors which mimics the basement membrane underlying epithelial cells and has been broadly used for assessing cell invasion, in vitro. The Matrigel used in this protocol was supplied by BD and was extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins including collagen IV, heparan sulfate proteoglycans, entactin, and nidogen. It also contains TGF-β and FGF, tissue plasminogen activator, and other growth factors which occur naturally in the EHS sarcoma. This protocol is only suitable for an invasion assay using serum as the chemoattractant. For growth factor mediated cell invasion assays, the protocol should be optimized and growth factor-depleted Matrigel is highly recommended.

The protocol primarily describes the method for Matrigel coating on CIM-Plates 16. In addition to the reagents specifically listed in this protocol for an invasion assay, other reagents and equipment are the same as those described in xCELLigence System Technical Note No. 3 – General Cell Migration Protocol Using the CIM-Plate 16.

1. Protocol

1.1 Cell Lines

HT-1080 cells (ATCC; Cat. No. CCL-121) were used in this protocol. If other cell lines will be used, the protocol may need to be optimized accordingly.

1.2 Reagents

- Matrigel (BD; Cat. No. 354234)
  Thaw the Matrigel on ice and aliquot to 100 µl volume in a 0.5 ml reaction tube. Store the Matrigel aliquots at -80°C. Re-freezing used Matrigel is not recommended.

  The concentration and quality of Matrigel provided by BD may vary from batch to batch. Therefore, for each new batch of Matrigel, it is highly recommended to perform a titration assay as described in the experimental example to test the quality and concentration of the new batch of Matrigel, and compare the result with the previous batches.

- HT-1080 cell culture complete media: DMEM (HyClone; Cat. No. 3002802), containing 10% Fetal Bovine Serum (FBS) (HyClone; Cat. No. SH 3007103), 1% Penicillin-Streptomycin solution (Mediatech; Cat. No. 300-002-CI), 1% MEM Nonessential Amino Acid solution (Mediatech; Cat. No. 25-025-CI)

- Serum-free medium (SFM): DMEM (HyClone; Cat. No. 3002802)

- TNS solution (Clonetics, Cat. No. CC-5002)

- 0.05% Trypsin/EDTA solution (GIBCO; Cat. No. 25300)

- Phosphate buffered saline (PBS, HyClone; Cat. No. 3002201)
## 2. Procedure

<table>
<thead>
<tr>
<th>Workflow Summary:</th>
</tr>
</thead>
<tbody>
<tr>
<td>This two-day assay protocol has been developed for continuous monitoring of cell invasion and migration on the RTCA DP Instrument for 24 hours.</td>
</tr>
</tbody>
</table>

### 24 hours before starting procedure:
- **Passage the cells; they should be 60 – 80% confluent for the experiment.**
- **Place pipette tips, reaction tubes, and the Upper Chamber (UC) of the CIM-Plate 16 in a +2 to +8°C refrigerator to cool.**
- **Transfer a tube of aliquoted Matrigel (see Reagents section of this protocol) from the -80°C freezer to a +2 to +8°C refrigerator.**

### Day 1:
- A. Coat the Upper Chamber with Matrigel.
- B. Assemble the CIM-Plate 16.
- C. Equilibrate at +37°C.
- D. Prepare cells.
- E. Add cells, equilibrate at room temperature and start measurement.

### Day 2:
- F. Stop the measurement and analyze the CI (Cell Index) curves to determine cell migration and invasion activity. Optionally, stain the migrated cells on the underside of the membrane and examine the stained cells under a microscope.
Day 1

A. Matrigel Coating of the Upper Chamber of CIM-Plate 16  Time: approx. 4 hours

1. Dilute the Matrigel with pre-cooled SFM on ice in pre-cooled reaction tubes. Approximately 1 ml of diluted Matrigel is needed to coat the UC of one CIM-Plate 16.

2. Dilute the Matrigel with cold SFM to separate ratios of 1:40 (1 Matrigel:40 SFM), 1:20, and 1:10 respectively. Maintain Matrigel solutions on ice at all times during the dilution process to avoid polymerization.
   - It is best to make a 1:10 dilution first and then perform serial dilutions to obtain 1:20 and 1:40 dilutions of Matrigel.

3. Add 50 µl of 1:10 Matrigel solution into the the center of the first four wells of the UC, according to Figure 1 and Table 1. Do not add Matrigel solution close to the wall. This may result in an uneven distribution of Matrigel due to surface tension and to formation of a meniscus.

4. Immediately remove 30 µl of Matrigel solution from the first four UC wells, leaving the remaining 20 µl of Matrigel solution to coat the surface of each well.
   - Do not wait until the whole plate is coated before removing the Matrigel.
   - This step is crucial to creating an evenly coated Matrigel layer in the wells. When removing the 30 µl of Matrigel, go as far down as possible into the well without touching the membrane and slowly withdraw the 30 µl of Matrigel. It is also important to exercise caution and not introduce any bubbles at this step. In the event of bubble formation, try using a dry tip to “pop” the bubble.

5. Repeat steps 3 and 4 with the remaining dilutions of Matrigel, according to Figure 1 and Table 1. Be sure to immediately remove the 30 µl of Matrigel from each group of four wells.

![Figure 1: Arrangement of three concentrations of Matrigel dilutions in the Upper Chamber of a CIM-Plate 16.](image)

6. Place the Matrigel-treated UC in the assembly tool and then place them together in a +37°C tissue culture incubator for 4 hours. It is important that the UC remains suspended and not be in direct contact with any surface, to avoid wicking of the unpolymerized Matrigel. Also, keep the UC covered with the lid to avoid contamination and evaporation.
B. CIM-Plate 16 Assembly  

**Time:** approx. 5 minutes

1. Fill each well in the lower chamber (LC) of the CIM-Plate 16 with 160 µl prewarmed media. To wells A1-G1 and A2-G2, add 160 µl of prewarmed 10% serum-containing media. To wells H1 and H2, add 160 µl of prewarmed SFM. Ensure that a meniscus is formed on each well after the well is filled with media.

<table>
<thead>
<tr>
<th>Well</th>
<th>Left Column (1), Lower Chamber</th>
<th>Left Column (1), Upper Chamber</th>
<th>Right Column (2), Lower Chamber</th>
<th>Right Column (2), Upper Chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:40 pre-coated</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:40 pre-coated</td>
</tr>
<tr>
<td>B</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:40 pre-coated</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:40 pre-coated</td>
</tr>
<tr>
<td>C</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:20 pre-coated</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:20 pre-coated</td>
</tr>
<tr>
<td>D</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:20 pre-coated</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:20 pre-coated</td>
</tr>
<tr>
<td>E</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:10 pre-coated</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:10 pre-coated</td>
</tr>
<tr>
<td>F</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:10 pre-coated</td>
<td>160 µl of 10% FBS medium</td>
<td>Matrigel 1:10 pre-coated</td>
</tr>
<tr>
<td>G</td>
<td>160 µl of 10% FBS medium</td>
<td>Uncoated</td>
<td>160 µl of 10% FBS medium</td>
<td>Uncoated</td>
</tr>
<tr>
<td>H</td>
<td>160 µl SFM</td>
<td>Uncoated</td>
<td>160 µl SFM</td>
<td>Uncoated</td>
</tr>
</tbody>
</table>

Table 1: Arrangement of Matrigel dilutions and media in Upper Chamber and Lower Chamber of CIM-Plate 16.

2. Assemble the CIM-Plate 16 (see xCELLigence System Technical Note No. 3 for detailed assembly instructions).

3. Add 30 µl SFM to the center of each well of the UC to cover the membrane surface. Do not introduce any bubbles, and do not allow the pipette tips to touch the membrane.

   - The volume of SFM is not critical here. The key is to ensure that the medium covers the entire bottom surface of the UC.

C. CIM-Plate 16 Equilibration at +37°C  

**Time:** approx. 60 minutes

1. Place the CIM-Plate 16 onto the RTCA DP Analyzer inside a +37°C incubator. The tapered corner of the CIM-Plate 16 should match to the tapered corner on the RTCA DP Analyzer. Incubate for 1 hour to allow the membrane surface to reach equilibrium with the media. During this 1 hour equilibration step, prepare the cells.

D. Cell Preparation  

**Time:** approx. 20 minutes

1. Cells should be passaged the day prior to the experiment and should be in the range of 60–80% confluence.

   - Like any other cell-based assay, the ultimate success of the invasion experiment using the CIM-Plate 16 depends on the quality and handling of the cells. It is imperative to critically review and follow the steps for cell maintenance and handling in order to ensure reliable and reproducible results. Also, it is important to note the passage number of the cells, as certain cell types can attain higher levels of background migration with increasing passage number.

   - Some cell types may possess an inherently high level of background migration; it may be important to serum-starve the cells prior to detachment. Cells can be serum-starved for 1 to 16 hours prior to detachment for migration assays. These conditions need to be determined empirically. Even a gradual decrease of serum concentration in several steps may be necessary.

2. Remove serum-containing medium from the flask and gently rinse the cell monolayer once with PBS.

3. Trypsinize cells by adding 0.5 ml of 0.05% Trypsin/EDTA solution per T-25 flask and leave the flask at +15 to +25°C or +37°C for 1–2 minutes. Do not over-trypsinize the cells. Check the degree of trypsinization under a microscope.

   - Cell migration and invasion are dependent on the expression and integrity of cell surface receptors such as integrins; it is important to minimize the time of protease treatment. Certain cell types can be sensitive to protease digestion and it may be important to explore alternative methods of cell detachment (i.e., using EDTA-based buffers).

4. Stop trypsinization by adding low serum media or TNS solution (Clonetics; Cat. No. CC-5002) at a ratio of 1:1.

5. Wash trypsinized cells once with SFM by centrifugation. For most cells 5 minutes at 800 × g is sufficient.

6. Gently resuspend the cell pellet in a few milliliters of SFM and count the cells under a microscope using a hemocytometer or using an automated cell counter. Adjust the concentration of the cell suspension to 2 × 10⁵ cells/ml, using SFM.
E. Background Measurement and Invasion Assay

**Time:** approx. 24 hours

1. After the 1-hour incubation step above is complete, start step 1 (1 minute and 1 sweep) in the RTCA Software to perform a background measurement.

2. Using a single channel pipette, add 100 µl of $2 \times 10^5$ cell/ml suspension (20,000 cells total) to each well of the UC.
   
   During addition of the cells the pipette tip should touch the surface of media already present in the well.
   
   Be aware that bubbles can be entrapped in the bottom of the well, forcing cells to migrate at the edge or block cell migration altogether. The best way to avoid bubble entrapment is to first slightly tilt the device at an angle, followed by insertion of the pipette tips all the way to the bottom of the well of the UC (avoiding the electrodes) and slowly ejecting the cell suspension. One way to check for possible bubble entrapment is to observe the level of the medium in the wells of the UC. If you added the same volume to each well, then the volume level in all the wells should be the same. If any wells contain air pockets or bubbles, then the volume level will be slightly higher than in the other wells. If you observe this immediately after addition of cell suspension, you can try carefully aspirating the cell suspension and reapplying it to the well. Alternatively, if this is observed later, it is better to avoid removal of the bubble and simply make a note of it in your laboratory notebook.

3. Leave the CIM-Plate 16 in the tissue culture hood at +15 to +25°C for 30 minutes after cell addition to allow the cells to settle down on the bottom surface of the UC.

4. Place the CIM-Plate 16 containing the cells in the RTCA DP Analyzer inside the incubator. Start Step 2 and take measurements for 24 hours at intervals of 15 minutes.
   
   It is important to place the CIM-Plate 16 in the same cradle unit that was used for background measurement. Each plate can have slightly different background impedance measurements, which can affect the coefficient of variation of the data.
   
   For most cell types, the length of the invasion assay is limited to anywhere from 16 to 24 hours. With the RTCA DP Instrument, you can monitor your invasion assay for as long as you like. The recommended schedule for Step 2 is 15 minute intervals for 100 sweeps.

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**DAY 2**

F. Data Analysis

1. Stop the measurement.

2. In the RTCA Software, average all the replicates on the Plot page.

3. Analyze the Cell Index (CI) curves. Optionally, export data to Microsoft Excel and calculate a Cell Invasion Index (CII, see TYPICAL RESULTS).
3. TYPICAL RESULTS

3.1 Invasion of HT-1080 cells through Matrigel on CIM-Plates 16

In this experiment, we tested HT-1080 invasion activity (20,000 cells/well) in the Matrigel-coated membrane with different Matrigel dilutions (or densities). As shown in Figure 2, the invasion kinetics of HT-1080 cells were different from the migration kinetics (without Matrigel coating), as evidenced by the delayed onset of Cell Index (CI) signals and a smaller CI slope on cell invasion curves. Furthermore, the invasion kinetics of HT-1080 cells showed a clear dose-dependency on Matrigel dilutions. The invasion signals (CI values) can be detected earlier and are higher in the lower-density Matrigel dilutions (1:40) than in the higher-density Matrigel dilutions (1:20 and 1:10). Based on the invasion kinetics of HT-1080 cells using the 1:20 Matrigel dilution, it took about 8 hours for the first cells to invade and migrate through the Matrigel layer.

There are multiple ways to analyze cell invasion data using the CIM-Plate 16. We have chosen to quantify the extent of invasion at different time points after cell seeding using a term called Cell Invasion Index (CII), which is the ratio of Cell Index of Matrigel-coated wells (invasion) to Cell Index of uncoated wells (migration) at a given time point. As shown in Figure 3, the CII is dependent on both the Matrigel concentration (with the most dilute concentration producing the higher CII) and time.
Appendix: Troubleshooting

The protocol described in this section does require some practice with CIM-Plate 16 migration in order to obtain reproducible data. For general troubleshooting information see xCELLigence System Technical Note No. 3.

⚠️ Particular attention should be paid to developing good techniques for Matrigel coating because it can be a source for increased variation in the experiment.

1. There is increased variation between the replicates:
   - Check the Matrigel to make sure it has not expired.
   - Be sure to allow the Matrigel to thaw overnight on ice.
   - Make sure to thaw a fresh vial of Matrigel each time. Do not re-freeze Matrigel.
   - Make sure all materials coming in direct contact with the Matrigel (including pipette tips, reaction tubes, CIM-Plates 16, and media) are kept cool at +2 to +8°C until ready to be used.
   - When covering the well with diluted Matrigel, make sure the gel covers the entire surface of the well.
   - Make sure, when removing 30 µl of Matrigel, that an equal volume of Matrigel is removed from every well.
   - Make sure no bubbles are introduced during the coating procedure.

2. No signal is detected in wells coated with Matrigel:
   - Make sure that the Matrigel is sufficiently diluted. For each new batch of Matrigel, it is imperative to perform a titration according to the protocol described in this document.

3. There is no difference in signal or CI dynamics between Matrigel-coated wells and uncoated control:
   - The Matrigel may be too dilute; try lowering the extent of dilution.
   - The Matrigel may not be sufficiently polymerized; it is important to allow the Matrigel to polymerize at +37°C in the CO₂ incubator. A humidified environment is required for optimal Matrigel polymerization.

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