RTCA DP Instrument Short Guide II: Cell Migration Assay in CIM-Plate 16 – *(November 2009)*

This short guide describes a typical cell migration experiment run on the D1 cradle of the RTCA DP Instrument.
A basic cell migration assay is described using HeLa cells with 10% Fetal Bovine Serum (FBS) as attractant in six replicates. The following is a recommended protocol meant to serve as a guideline. It may be modified to fit individual protocols for different cell lines or other chemoattractants.

1. Assembly of the CIM-Plate 16

1. Remove both upper and lower chambers of the CIM-Plate 16 from the package. Place the CIM-Plate 16 Assembly Tool inside the tissue culture hood with the blue spots away from you. Load the lower chamber into an individual pocket of the assembly tool and ensure that the lower chamber sits flat inside.
   - *There is only one correct orientation for the lower chamber inside the tool. The blue spot on the lower chamber should be positioned at the corresponding location of the blue spot on the tool.*
2. Fill each lower chamber well with **160 µl** media (containing 10% FBS or serum-free as control) using an 8 channel pipette.
   - *Ensure that a clearly defined meniscus is formed in each well after the well is filled with media. To minimize bubble formation in the lower chamber, do not introduce bubbles while pipetting the media.*
   - *160 µl recommended here is based on a calibrated pipette, while exact volume for pipetting into the lower chamber may need to be optimized by the user, depending on the pipette calibration condition. The optimization should be based on using the lowest volume without bubbles in the lower chamber after the lower chamber is assembled with the upper chamber.*
3. Turn the assembly tool 90 degrees counter-clockwise. Place the upper chamber onto the lower chamber with the sensor surface facing down and the blue spot on the upper chamber on the same side as the lower chamber. Push the upper chamber downwards to lock the upper and the lower chambers together.
   - *Two "click-clack" sounds should be heard to ensure that the chambers have locked together properly.*
4. Add 25-50 µl serum-free media to each well of the upper chamber to cover the membrane surface.
   - *During media addition, do not introduce any bubbles and avoid touching the membrane with the pipette tips.*
   - *The serum-free media volume is not critical here. The key is to ensure that media covers the entire lower surface of the upper chamber.*

2. Equilibration of the CIM-Plate 16

Load the CIM-Plate 16 onto the RTCA DP Analyzer, such that the edge-cut corner on the CIM-Plate 16 should match to the edge-cut corner of the pocket in the RTCA DP Analyzer inside the incubator. Wait one hour to allow the CIM-Plate 16 membrane surface to reach an equilibrium with media.

3. Start of the RTCA Software 1.2

1. Start the RTCA Software by double-clicking the *RTCA Software* icon on the desktop.
2. Click on the *Plate Window* button to select the D1 Plate Window.

4. Set-up of the *Exp Notes* Page

Click on the *Exp Notes* tab in the RTCA Software. Enter the information for this experiment (*e.g.*, experiment name, experiment purpose, device part no.) in the appropriate spaces.

5. Set-up of the *Layout* Page

1. Click on the *Layout* tab in the RTCA Software. Enter information into appropriate boxes, and click *Apply*. For example: Highlight well A1 to H1, *Cell Type* - HeLa; *Cell Number* - 40,000. Then, highlight well A2 to H2, *Cell Type* - HeLa; *Cell Number* - 20,000.
2. Highlight well A1, A2 and H1, H2, *Compound Name* - SF_Control (Serum-free control) and click *Apply*.

- *Cradle D2 or D3 (well A3 to H6) can be used for other cell lines as described above, or for different experimental applications.*

6. Set-up of the *Schedule* Page

1. Click on the *Schedule* tab in the RTCA Software.
2. Click on the *Add a Step* icon. *Step 1* appears in the *Step* column. Step 1 is the background step.
3. The *Step Status* column should state *IDLE* for step 1. The *Sweeps* and *Interval* should automatically be set to ***1*** and ***1.00*** minute.
4. Click on the *Add a Step* icon to create step 2.
5. Enter ***100*** in the *Sweeps* box and ***15.00*** in the *Interval* box. Choose *minute* as the unit definition. Click *Apply*.
6. Once step 2 is initiated, the RTCA DP Instrument will monitor cell migration for up to **25 hours** (100 sweeps every 15 minutes).

7. Background Measurement

1. After one hour equilibration with media, start *step 1* (1 minute with 1 sweep) to perform background measurement.
2. Once the measurement is completed, the bottom-left of the main window displays the message *Ready for Next Step. Please Click Next Step to start.* Click on the *Message* tab to read any other messages with respect to background or connection problems.

8. Cell Preparation

1. Cells of interest should be passaged one day before the experiment and should reach 60-80% confluence.
   It is recommended that cells used for cell invasion/migration assays should be passaged less than 20 times. The maximum passage number may be different from different cell types and may be smaller than 20 for primary cell cultures.
2. Remove serum containing media from the flask as much as possible, and gently rinse the cell monolayer with PBS once to wash off the remaining serum containing media.
3. Trypsinize the cells by adding 0.5 ml of 0.05% Trypsin/0.02% EDTA solution per T25 flask and leave the flask at room temperature or +37 °C for 1-2 minutes.
4. Stop trypsinization by adding 10% FBS containing media or TNS solution (*e.g.*, from Clonetics) at a 1:1 ratio.
5. Wash trypsinized cells once with FBS-free medium and resuspend after centrifugation.
6. Count cells and prepare cell suspension in FBS-free media at the concentration of 4×10^5 cells/ml and 2×10^5 cells/ml, respectively.

9. Cell Addition to CIM-Plate 16

1. Unlock the D1 cradle by gently pushing the press-button with the thumb whilst holding the cradle grip with other fingers. Remove the CIM-Plate 16 from the D1 cradle.
2. Add 100 µl cell suspension of 4×10^5 cells/ml to well A1 to H1 of the upper chamber and cell suspension of 2×10^5 to well A2 to H2. The final cell numbers per well should be 40,000 and 20,000, respectively.

10. Equilibration of the CIM-Plate 16 at Room Temperature for Cell Sedimentation

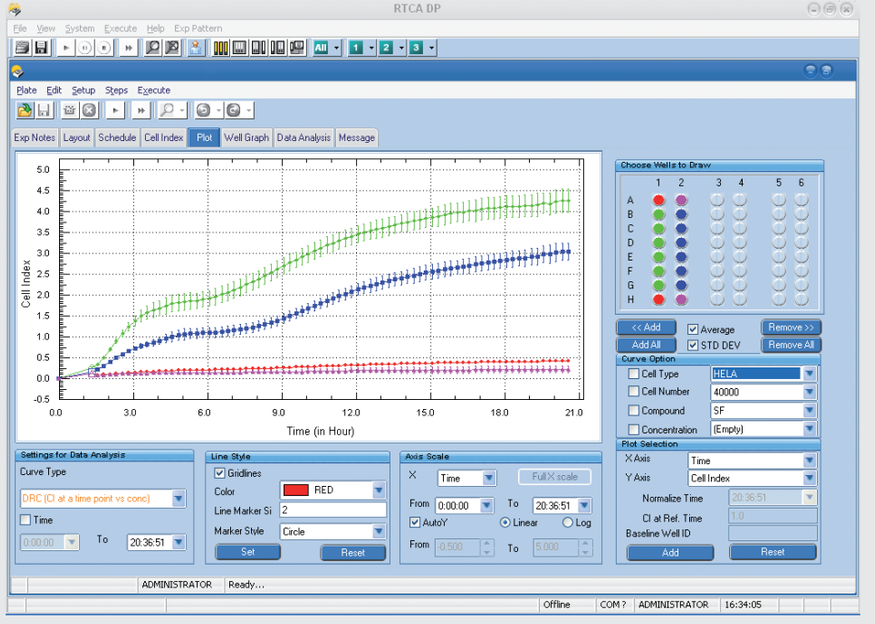
Leave the CIM-Plate 16 in the tissue culture hood at room temperature for 30 min after cell addition to allow the cells to settle down to the upper side of the membrane located in the bottom of the upper chamber.

11. Start of the Measurement

1. Load the CIM-Plate 16 into the RTCA DP Analyzer inside the incubator as described above (see **2. Equilibration of the CIM-Plate 16**). Close the incubator door.
2. A *Scan Plate* is performed automatically. Open the *Message* tab to check whether *Scan Plate* was successful. The following message should be displayed: *Plate scanned. Connections ok.*
3. Start step 2 by clicking the *Start Step* button. The RTCA DP Instrument will now automatically monitor the cells every **15 minutes** for **100 repetitions**.

12. Check and Plot the Cell Index Data to determine Cell Migration Activity

1. On the second day, (*e.g.*, after 18~20 hours), check and plot the Cell Index in the *Plot* page of the RTCA Software.
2. Click *Add All* to show data for every well included in the experiment.
3. Activate the *Average* and *STD DEV* boxes to display the averages and corresponding standard deviation error bars of the respective replicates.
4. Analyze the Cell Index (CI) curves. The positive migration signals should meet the criteria of averaged CI ≥ 2 fold standard deviation of the Cell Index.
   This criteria of the average Cell Index as positive result is cell type specific. The user should determine whether a Cell Index signal is positive based on specific assay conditions.



Example of results from a cell migration experiment using 40,000 (green line) or 20,000 (blue line) HeLa cells with 10% Fetal Bovine Serum (FBS) as the chemoattractant, or without FBS as a control (40,000 HeLa cells in red and 20,000 HeLa cells in magenta line)

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