RTCA DP Instrument Short Guide I: Cell Titration in E-Plate 16 — *(January 2013)*

For illustration purposes, this short guide describes a cell titration experiment run on the D1 cradle of the RTCA DP Instrument.
A typical cell titration and growth experiment on the RTCA DP Instrument is performed in duplicate. The following is a recommended protocol meant to serve as a guideline.
It may be modified to fit individual experimental workflows.

1. Add Culture Media to the E-Plate 16

1. Add **100 µl** of cell culture media to each well using a Multipette.
2. Leave the E-Plate 16 in the tissue culture hood at room temperature to ensure that the culture media and E-Plate surface achieve equilibrium.
   Do not touch the electrical contacts of the E-Plate 16 and always wear protective, powder-free safety gloves!

2. Preparation of Cells

1. Obtain freshly split cells of the cell line(s) of interest (**75% to 85%** confluence).
2. Wash cells with PBS and detach by adding the appropriate volume and concentration of Trypsin-EDTA solution.
3. Dilute the detached cells with cell culture media and spin down the cells.
4. Resuspend the cells in fresh cell culture media.
5. Count the cells.
6. Adjust the cell concentration to **400000 cells/ml**. Perform **2-fold serial dilutions** in **6** additional tubes (preferably 50 ml conical tubes) to obtain cell densities of **200000**, **100000**, **50000**, **25000**, **12500** and **6250 cells/ml**.
   In the proposed setup, it is possible to monitor cell growth and proliferation for 1 cell line (7 concentrations, duplicates) in one E-Plate 16.
   Alternatively, serial dilutions can also be performed directly in the E-Plate 16 (in step 9 2).

3. Start the RTCA Software

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

4. Set up the Exp Notes Page

1. 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 the Layout Page

1. Click on the *Layout* tab in the RTCA Software.
2. Highlight the wells A1 to G2. Enter the name of the cell line (*e.g.*, HeLa) in the *Cell Type* box, the highest cell number (**40000**) in the *Cell Number* box and checkmark the radio button for *Cell Number Titration*.
3. Enter the following information in the appropriate boxes: *Titration Factor* **2**, *Repetition* **1**, *Along* **Column**, *High From* **Top** and click *Apply*.
   The different cell numbers per row (**40000**, **20000**, **10000**, **5000**, **2500**, **1250**, **625**) are calculated automatically.
4. Highlight the wells H1 to H2. Enter *"Cell Line" Control* (*e.g.*, HeLa Control) in the *Cell Type* box and click *Apply* to register the information in the RTCA Software.
   Cradle D2 (well group A3 to H4) and D3 (well group A5 to H6) can be used for cell titration of a 2nd and 3rd cell line as described above, or for different experimental purposes.

6. Set up the Schedule Page

1. Click on the *Schedule* tab in the RTCA Software.
2. Click on the *Add a Step* icon or alternatively go to the top of the page in the *Steps* drop-down menu and click *Add a Step*.
   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 or alternatively go to the top of the page in the *Steps* drop-down menu and click *Add a Step* to create step 2.
5. Enter ***200*** in the *Sweeps* box and ***30*** in the *Interval* box. Choose *minute* as the unit definition. Click *Apply*.
   Once step 2 has been initiated, the RTCA DP Instrument will monitor cell growth and proliferation for up to **100 hours** (200 sweeps × 30 minutes).

7. Insert the E-Plate 16

1. Insert the E-Plate 16 (from step 1 (1)) front end into the D1 cradle pocket of the RTCA DP Analyzer.
2. Make sure that the E-Plate 16 is properly aligned without any tilting. Let go off the thumb from the press button and the fingers from the cradle's grip, the clamp plate will lock the E-Plate 16 in the horizontal position.
3. Close the door of the cell culture incubator.
4. An automatic *Scan Plate* is performed to check for proper contact after the E-Plate 16 has been inserted and the lock handle locked.
5. Open the *Message* tab of the RTCA Software to check whether *Scan Plate* was successful. The following message should be displayed: *Plate scanned. Connections ok.*

8. Perform Background Measurement

1. Click *Start a Step* icon to begin the experiment. Background impedance of each well is measured during step 1.
2. After completion of the measurement the bottom-left of the main program window displays the message *Ready for Next Step. Please Click Next Step to start.*
3. Click on the *Message* tab to read any other messages with respect to background or connection problems.

9. Remove the E-Plate 16 and Add Cells to the Wells

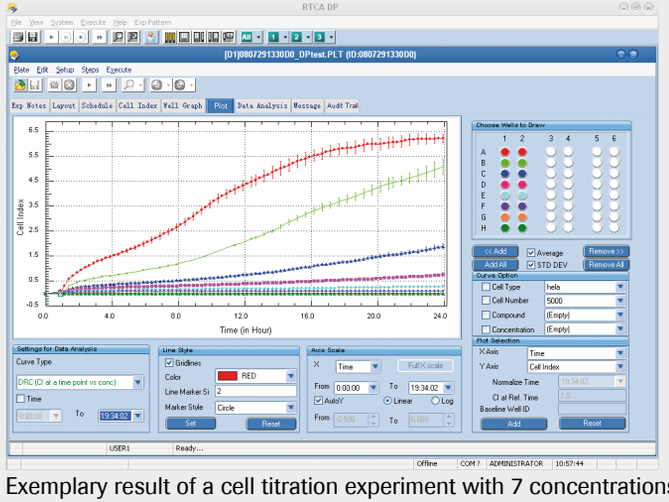
1. Unlock the D1 cradle by gently pushing the press-button the thumb whilst holding the cradle grip with other fingers. Remove the E-Plate from the D1 cradle.
2. Add **100 µl** of cell suspension (from step 2 (6)) in duplicate to the appropriate wells. Make sure the cells are well suspended. Add **100 µl** of cell culture media to each well of the cell line controls (row H).
   - Prior to transferring the cells to the E-Plate 16, make sure the cells are evenly suspended by shaking the cells in the tube. Transfer the cell suspension to a reagent reservoir and use a Multipette for pipetting cells uniformly to the appropriate wells.
   - The cell line control (row H) reports any effects relating to cell culture media addition and the removal/insertion of the E-Plate 16.
3. Leave the E-Plate 16 in the tissue culture hood for **30 minutes at room temperature**.
   - This incubation time is necessary to allow the cells to settle in an evenly distributed pattern at the bottom of the wells.

10. Reinsert the E-Plate 16 and Start Step 2

1. Insert the E-Plate 16 as described above (step 7). Close the incubator door.
2. A *Scan Plate* is performed automatically.
3. Open the Message tab to check whether *Scan Plate* was successful. The following message should be displayed: *Plate scanned. Connections ok.*
4. Start step 2 by clicking the *Start Step* button. The RTCA DP Instrument will now automatically monitor the cells every **30 minutes** for **200 repetitions**.
   - During the experiment, a countdown to the next sweep start time is displayed on the bottom left of the main program window.
   - Once the experiment has been initiated, avoid multiple opening and closure of the incubator door.

11. Check and Plot the Cell Index Data

1. During or after the experiment, check the *Plot* page of the RTCA Software.
2. Plot the Cell Index of a well as a function of time by highlighting the well and clicking *<< Add*. To add another well, repeat the process.
3. Click *Add All* to show data for every well included in the experiment.
4. Activate the *Average* and *STD DEV* boxes to display the averages and corresponding standard deviation error bars of the respective replicates.



Exemplary result of a cell titration experiment with 7 concentrations in duplicates.

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