




## RTCA SP Instrument Short Guide I: Cell Titration - (October 2008)

A typical cell titration and growth experiment on the RTCA SP Instrument is performed in quadruplicate. 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 96

- 1 Add **100 µl** of cell culture media to each well using a Multipipette.
- 2 Leave the E-Plate 96 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 96 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 3 cell lines (7 concentrations, quadruplicates) in one E-Plate 96.
  -  Alternatively, serial dilutions can also be performed directly in the E-Plate 96 (in step 9 **2**).



### 3. Start the RTCA Software

- 1 Start the RTCA Software by double-clicking the *RTCA Software*  icon on the desktop.





### 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 G4. 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 H4. Enter "*Cell Line*" *Control* (e.g., HeLa Control) in the *Cell Type* box and click *Apply* to register the information in the RTCA Software.
  -  Well groups A5 to H8 and A9 to H12 can be used for cell titration of a 2<sup>nd</sup> and 3<sup>rd</sup> 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 SP Instrument will monitor cell growth and proliferation for up to **100 hours** (200 sweeps × 30 minutes).




## 7. Insert the E-Plate 96

- 1 Insert the E-Plate 96 (from step 1 **1**) front end into the cradle pocket of the RTCA SP Station.
- 2 Make sure that the E-Plate 96 is properly aligned without any tilting. Lift the lock handle of the RTCA SP Station so that the two bearings of the clamp plate fit into the grooves of the lock handle. Then push the lock handle downward to the horizontal position.
  -  The green light is illuminated when the E-Plate 96 is correctly positioned.
- 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 96 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* 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 96 and Add Cells to the Wells

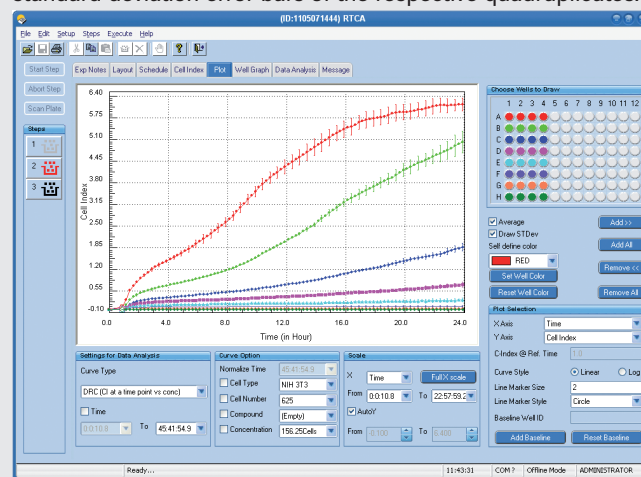
- 1 Remove the E-Plate 96 from the RTCA SP Station by gently releasing the lock handle upward. The green light goes out.
- 2 Add **100 µl** of cell suspension (from step 2 **6**) in quadruplicate to the appropriate wells. Make sure the cells are well suspended. Add **100 µl** of cell culture media to each well of the cell line controls (row H).
  -  Prior to transferring the cells to the E-Plate 96, 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 96.
- 3 Leave the E-Plate 96 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 96 and Start Step 2

- 1 Insert the E-Plate 96 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 SP 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 *Draw STDev* boxes to display the averages and corresponding standard deviation error bars of the respective quadruplicates.



Exemplary result of a cell titration experiment.