# RTCA SP Instrument Short Guide II: Basic Recommendations – *(December 2008)*

## 1. General recommendations:

1. Always **put on gloves** before handling the E-Plates 96 and the RTCA SP Station.
2. Do **not touch the RTCA Contact Pins and the Contact Pads**.
3. Do not start a background measurement until **all wells are filled with medium or PBS**. It is especially important to fill the same solution (medium or PBS) into all of the 4 wells that are connected to one contact pad (A1-D1, E1-H1, A2-D2, E2-H2…..).

## 2. Installation handling:

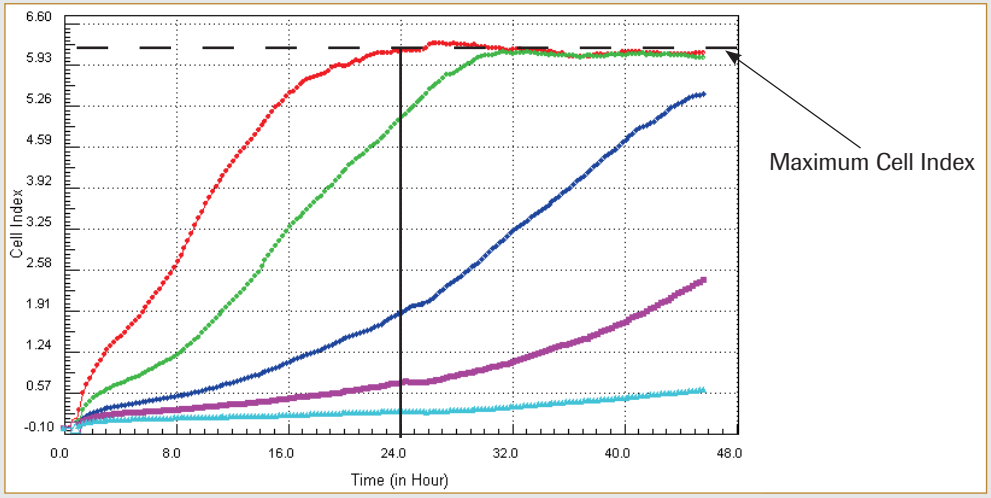
1. Do not place the incubator used for the RTCA SP Station next to (*e.g.* on the same bench) a centrifuge or another instrument that can potentially produce vibrations.
2. Check for condensation in the incubator – if condensation is present do not put the RTCA SP Station into the incubator – the incubator needs to be checked.
3. **Clean the RTCA SP Station** (wipe the RTCA SP Station with 80 % ethanol excluding the pins).
4. **Equilibrate the RTCA SP Station** in the incubator for **at least 2 hours** - do not use the RTCA SP Instrument if condensate has formed on the RTCA SP Station after these 2 hours. To avoid excessive condensation make sure that the RTCA SP Station is at least at room temperature before placing it into the incubator.
5. **Perform a Resistor Plate verification** if the RTCA SP Instrument is newly installed or has not been used for a long period.

## 3. Experimental design:

1. It is recommended that **each sample** is set up **in triplicate**.
2. For compound addition **always use the following controls**: cells plus solvent without compound (vehicle control), as well as cells plus medium only (without any treatment). In addition, medium only, without cells, is recommended at least for the first assays.

## 4. Planning the first experiment with the system or a new type of cells:

First measure the proliferation of a cell dilution series. Then plot the growth curves **to find out** how many cells should be seeded and **at which time point** and **Cell Index**, respectively, **the cells should be treated** (be aware that the area of an E-Plate well is 0.20 $cm^2$, which is smaller than that of a standard 96 well plate). For most compounds the cells should be treated at **1/3 or 1/4 of the maximum CI**.




10000 HeLa cells
5000 HeLa cells
2500 HeLa cells
1250 HeLa cells
625 HeLa cells

Exemplary result of a cell titration experiment.

Example:
If the cells should be treated 24 hours after seeding, then it is recommended to use approximately 2000-2500 cells/well, because 24 h after seeding 2000-2500 cells/well would reach about 1/3 of the maximum CI.

## 5. Starting an experiment – minimum software entries:

1. Start the RTCA SP Software (if the software is still running – click *File* and then *Release*) – a new blank experiment is automatically opened.
2. Go to *Layout* page and select all wells and click *Apply* – the system will measure the CI only in the applied wells. It is not necessary to enter the number of cells, compounds and the concentrations of the compounds at this point – this information can be added after pausing the experiment or after the whole experiment is completed.
3. Fill in the *Schedule* page – the first step (background measurement) should not be changed.
4. **Fill all wells with media** (usually **50–100 µl**).
5. Insert the E-Plate 96 front end into the cradle pocket of the RTCA SP Station.
6. Check the *Message* page to confirm that all connections are OK.
7. **Click *Start step*** to initiate step 1 (background measurement) – check to confirm that all wells are OK.
8. **Add** the appropriate volume and dilution of **cells**. **Incubate 30 min at room temperature**.
9. Re-insert the E-Plate 96 as described above (step 5).
10. **Click *Start step*** to initiate step 2.

## 6. Experimental handling:

1. Use **50–100 µl** of medium for the background measurement (always the first step).
2. Do not use cold medium – the temperature of the medium should **be between RT and 37°C**.
3. Directly before **filling the cell suspension** into the wells – **mix the cell suspension** by pipetting up and down **approximately 10 times** – it is important to pipette continuously and quickly – use multichannel pipettes – ideally we would recommend to use electronic multipipettes - mix again before every pipetting step in case regular multichannel pipettes are used.
4. The **amounts of cells and media** should be adjusted for the duration of the experiment. Take into account the total time of the experiment, the number of cells and the media supply (**the ideal quantity is usually 200 µl**). For some experiments (*e.g.* if an end-point assay is to be performed in the E-Plate) it is more convenient to let the cells grow in 100 or 150 µl.
5. If serial **dilution of the cells** is performed, **mix** by pipetting up and down **at least 10 times** before pipetting the next dilution.
6. **After seeding the cells** – **leave the plate for 30 min under the flow box** to allow the cells to sediment homogeneously – exception: cell adhesion experiments.

## 7. Compound addition:

1. Ideally the compound **volume to** should be between **2–5 µl**. Add compounds up to a maximum of 10 µl. The **maximum volume of liquid per well** should not exceed **210 µl** (*e.g.*, let the cells grow in 200 µl medium and add compounds in 5 µl).
2. **DMSO concentration:** the concentration of DMSO should be as low as possible **[0.05–0.1 % (maximum: 0.2 %)]** - all wells (all compound concentrations) should have the same proportion of DMSO:
   a. Start with a dilution in DMSO.
   b. Continue with a second dilution in medium.
3. **PBS concentration:** should also be as low as possible – but for PBS a second dilution step is not necessary in most cases.
4. **Do not mix inside the well after adding of a compound to the cells.**

## 8. Scan Plate:

After each scan plate (scan plate is performed automatically each time an E-Plate is inserted into the RTCA SP Station) check to confirm that all wells are OK (*Message* and *Cell Index* page) – otherwise:

1. Open the RTCA SP Station, take the E-Plate 96 out of the RTCA SP Station and reinsert.
2. If the connection problem still exists – take the E-Plate out of the RTCA SP Station again and clean the E-Plate with a fiber-free tissue. Reinsert the E-Plate into the RTCA SP Station.
3. If the problem remains – clean the RTCA SP Station (see cleaning procedure in the RTCA SP Instrument Operator's Manual).

## 9. Cloning an experiment:

If you want to use the same, or a similar, setting as used in a previous experiment – a complete experimental software setup or just the page of interest *e.g. Layout* or *Schedule* page can be imported:

1. Start the software or click *File* and click then *Release* to open a new blank experiment.
2. Click *Setup* and choose *Clone Experiment* or *Clone Individual Page*.
3. Select the experiment you want to clone.

For a comprehensive list of warnings, precautions, important notes and cleaning operations of the RTCA SP Instrument please refer to the RTCA SP Instrument Operator's Manual and the RTCA Software Manual.

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