

1. General Recommendations:

- 1 Always **put on gloves** before handling the E-Plate L8 and the iCELLigence station.
- 2 **Do not touch the iCELLigence contact pins or the contact pads**
- 3 Do not start a background measurement until **all wells are filled with media or PBS**. It is especially important to fill the same solution (medium or PBS) into all of the 8 wells.

2. Installation and Handling:

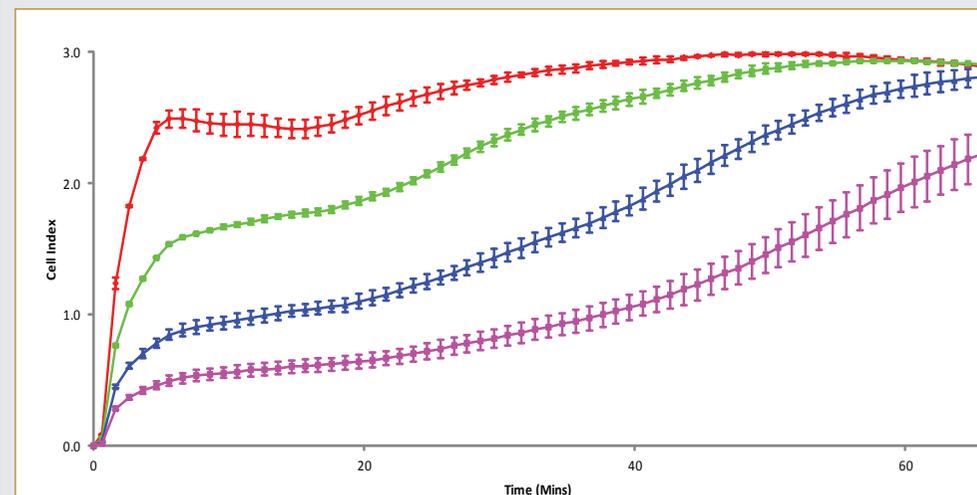
- 1 Do not place the incubator used for the iCELLigence Station next to (*e.g.* on the same bench) a centrifuge or another instrument that can potentially produce vibrations.
- 2 Check for condensation in the incubator – if condensation is present do not put the iCELLigence station into the incubator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

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 L8 well is 0.64 cm², which is much larger 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**.



45,000 HeLa cells
 22,500 HeLa cells
 11,250 HeLa cells
 5,625 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 10,000-12,000 cells/well, because 24 hours after seeding 10,000-12,000 cells/well would reach about 1/3 of the maximum CI.

5. Starting an Experiment – Minimum Software Entries:

- 1 Connect to the correct iCELLigence Station under the Apple iPad Settings application in the Wi-Fi section.
- 2 Start the iCELLigence Software on the iPad (If the software is still running – click the Home button  and then New Experiment) – a new blank experiment is automatically opened.
- 3 Select the desired Assay Type from the given list or Other Assay if none apply.
- 4 Check the Procedure page to determine if the Steps are sufficient for the assay involved. Add or edit the Steps if necessary. **The first Step (background measurement) should not be changed.**
- 5 Fill in the Layout with the desired cell and compound information. 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 entire experiment is completed.
- 6 **Fill all wells with media** (usually **150-200 µL**).
- 7 Insert the E-Plate L8 front end into the cradle pocket of the iCELLigence Station.
- 8 **Click the Start button** to initiate Step 1 (background measurement).
- 9 **Add** the appropriate volume and dilution of **cells. Incubate for 30 minutes at room temperature.**
- 10 Re-insert the E-Plate L8 as described above (step 6).
- 11 **Click the Start button** to initiate Step 2.

6. Experimental Handling:

- 1 Use **150-200 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) and to mix again before every step.
- 4 The **amount 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 450 µL**). For some experiments (e.g. if an end-point assay is to be performed in the E-Plate L8) it is more convenient to let the cells grow in 450 to 500 µL.

- 5 If a 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** into the E-Plate L8, **leave the plate for 30 minutes under the tissue culture hood** to allow the cells to sediment homogeneously. (Exception: Cell Adhesion experiments)

7. Compound Addition:

- 1 Ideally the compound **volume to add** should be between **25-50 µL**. Add compounds up to a maximum of 50 µL. The **maximum volume of liquid per well** should not exceed **650 µL** (e.g. let the cells grow in 600 µL medium and add compounds in 50 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 compounds to the cells.**

8. Scan Plate:

Upon the initial loading of the E-Plate L8 into the iCELLigence station, a message will appear on the screen notifying the user if there are any defective wells. If there are none present, the status of the experiment will read “StandBy.” If a defective well is detected:

- 1 Remove the E-Plate L8 from the iCELLigence station.
- 2 Clean the contact pads below the E-Plate L8 to make sure there is no dust or debris that would otherwise interfere with the electrical readings.
- 3 Gently clean the contact pins located on the iCELLigence station in the cradle pocket that houses the E-Plate L8.
 - a. **CAUTION:** Do not bend or remove the pins when cleaning. Gently scrub to ensure efficient cleaning of the pins without damaging the station.
- 4 If the defective well persists, disable the measurement of the well during the experiment (the ability to disable wells is located under the Layout tab – “Well Property”), and contact your local ACEA Biosciences, Inc. representative.

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 *Template* can be saved and imported:

- 1 To save a template: Start the iCELLigence software and click the Save Button  on the top right of the screen.
- 2 Select whether to save the settings as a Protocol (saves procedure settings) or a Template (saves procedure and layout settings).
- 3 Enter a name for the file and click Save.
- 4 To load a template: Start the iCELLigence software and click New Experiment.
- 5 Under the correct assay, select *User Defined Protocol*.
- 6 From the drop down menu, select the correct saved protocol or template.

10. Resuming an Experiment:

Before an experiment is resumed, it is important to make sure that the **iPad device is connected (via WiFi)** to the **correct iCELLigence station**. To determine the correct station, match the serial key located on the unit with that of the WiFi signal under the Settings application.

- 1 Start the iCELLigence Software.
- 2 On the Home Page, click Resume Experiment.
- 3 Select the experiment to be resumed from the list, and click OK.
- 4 Click on the WiFi Button  located on the top right to check and determine which iCELLigence station the iPad is currently connected to.
- 5 In general, a warning message will appear notifying the user if the iPad is not currently connected to the correct station.

11. Data Export

Export of data should be performed at the end of the experiment to ensure that all time points measured are included in the final file. In order to use this feature, the **Apple Mail application must be set up and configured**.

- 1 Start the iCELLigence Software and Resume the experiment if it is not already running.
- 2 Click the *Data Exporter Button*  located on the top right of the screen.
- 3 Select the data file you wish to export and click *Export*.
- 4 An email draft will show: Enter the email address the data will be exported to and any relevant information in the body of the email. Click *Send*.
- 5 To export the plot graph or table, click and hold (long-click) the graph or table.
- 6 Select *Export Chart and Data* if exporting a plot, or select *Export Data* if exporting a table.
- 7 The file is sent electronically via email and is made available to download on a separate computer for analysis.

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