Troubleshooting of Edge Well Effects in E-Plate 96
Troubleshooting of Edge Well Effects in E-Plate 96:

Under certain conditions (e.g., extremely low cell numbers) experimental data can vary between wells in the center and wells on the edge of E-Plates 96 (so-called “edge well effect”). This is also a commonly known phenomenon of conventional microtiter plates.

If you observe such effects in your experiments or effects detrimental to cell viability in general, please first address the following questions and comments to minimize edge well effects.

1. Was the E-Plate 96 left outside of the incubator for 30 min after cell addition to allow the cells to attach?

If this incubation step after cell addition is omitted, there is an increased risk of microcirculation inside the wells, especially at the edge wells. This circulation can lead to an uneven distribution of cells across the well bottom, producing a changed Cell Index (CI) in the edge wells compared to inner wells (see also: Lundholt BK, Scudder KM, Pagliaro L (2003) A simple technique for reducing edge effect in cell-based assays. J Biomol Screen 8, 566-570).

Example:

10,000 HT1080 cells per well were seeded into an E-Plate 96 and grown for 3 days. Please note that edge wells (blue) of samples not incubated at room temperature after seeding exhibit a different curve shape compared to inner wells (cyan).

The uneven distribution of cells when the 30 min incubation step has not been performed is sometimes seen after the experiment, when cells are stained using crystal violet or other dye (see right part of the following figure).

Columns 1 to 6: With 30 min incubation at room temperature after cell seeding (red = edge wells, green = inner wells).

Columns 7 to 12: Without 30 min incubation after cell seeding (blue = edge wells, cyan = inner wells).
2 Were all media prewarmed before usage (at least to room temperature) or were media/solutions used directly from the refrigerator?

Cell culture media and solutions should always be brought to room temperature before pipetting. If they are cold, equilibration to environmental temperatures will occur inside the wells. Due to the design of microtiter plates, this process will proceed with different kinetics between inner and outer wells.

3 Does the observed edge well pattern correlate with the order you pipetted into the E-Plate 96?

Multichannel pipettes can exhibit a slightly changed performance at different pipetting positions, especially the outer pipette positions. This may also be the case if one of the pipette tips is not firmly attached to the pipette. If for example channel no. 5 of an 8-channel pipette is slightly inaccurate, this will be reflected in changed CI values of the RTCA experiment (row E in the example).

Example:

5,000 HT 1080 cells per well were seeded into an E-Plate 96 and grown for 3 days:

Columns 1 to 6 (blue) with inaccurate channel 5 (row E, red); columns 7 to 12 (green) with correct pipette.
Were the cells and solutions mixed and diluted correctly?

Depending on the pipetting scheme used for the E-Plate 96, insufficient mixing or incorrect dilution of cells or substances can create edge well effects that are clearly an experiment handling issue.

Example:

10,000 HT 1080 cells per well were seeded into an E-Plate 96. Columns 1 to 4 were pipetted correctly, whereas columns 5 to 12 were pipetted with 1 min delay each without intermediate resuspension leading to sedimentation of cells and less cells seeded into wells.

For how long were the cell cultures maintained: ≤ 2 days, ≤ 1 week, > 1 week? How often was the cell culture media changed?

When running experiments for several days or weeks, it is necessary to partly exchange the culture media every other or every third day to maintain proper growth conditions for cells. Increased evaporation in outer wells and not renewing the media periodically increases the risk of edge well effects.

Do not mix after addition of new media; add media on top of the remaining culture media carefully without touching or disturbing the cells.

Example:

Proliferation curve of 5,000 H460 cells per well for 20 days (480 hours):
Proliferation curve of 5,000 H460 cells per well for 20 days (480 hours). Presentation of edge wells compared to the more inner wells:

Red/orange: With media change; Green/dark green: Without media change. Arrows indicate timepoints of culture media exchange. Red + dark green: Inner wells; Orange/green: Edge wells. In addition to an overall decrease in CI after 5 days, CI values start to differ between edge and inner wells of samples in which the media is not exchanged.

How many cells were seeded in the experiment? Is this cell number at the lower end of detectability?

As a general rule, edge well effects tend to be more prominent at lower initial cell density. If you obtain edge well effects, we recommend using a cell density as high as possible, but still appropriate to the experimental purpose and setup. For proper growth, cells require a minimum number of neighboring cells within each well. If the cell density is at the border or below this value, proper growth may only occur in some replicates, and not in others.

In order to determine the optimum cell density, always first perform a cell titration experiment.

Example:

500 (left) or 5,000 (right) HT1080 cells per well were grown for 5 days. Maximum CI value for these cells was approx. 4.5. As a general recommendation, compound addition should proceed at ~ 1/3 of the maximum CI. For this reason, replicate variation was assessed at a CI = 1.5:

Time for obtaining CI = 1.5 with 500 cells per well (left) was ~102 h, exhibiting a standard deviation of ±26.9%. Time for obtaining CI = 1.5 with 5,000 cells per well (right) was ~34 h, with a standard deviation of ±9.3%. Red: Inner wells; Blue: Edge wells.
Do you know if your cell type is susceptible to environmental changes?

Individual cell types react differently to environmental changes such as temperature, pH, CO₂ concentration, osmolarity, etc. When using a more sensitive cell type, the risk of edge well effects can increase, since these changes may be more prominent in the outer wells of an E-Plate 96.

Example:

In a proliferation experiment four different types of cell lines were grown. After ~16 h the E-Plate 96 was removed from the RTCA SP Station and the incubator to examine the cells under a microscope. Afterwards the E-Plate 96 was immediately returned to the RTCA SP Station and measurements were resumed.

The short shift in environmental conditions had different effects on the CI values of each cell line: HEK293 cells (blue) show a sharp transient decrease, whereas HepG2 (orange) and NIH3T3 cells (green) exhibit a transient increase in CI. HeLa cells (red) remain unaffected by this environmental change. n=24

What is the total volume you use for each well: 50 µl, 100 µl, 150 µl, 200 µl?

To assure optimal physiological conditions for cells, perform experiments using a high volume of liquid in each well (200 µl). A major factor influencing physiological conditions is evaporation from wells. This effect is more significant the lower the reaction volume in the wells is. Edge wells are known to have an elevated risk for evaporation.

Example:

5,000 HT1080 cells per well were seeded into an E-Plate 96 and grown for 90 hours. Different wells contained different amounts of culture media. After 2-3 days, the detrimental effects of low media volumes become apparent:

Red: 50 µl per well; Green: 100 µl per well; Blue: 150 µl per well; Pink: 200 µl per well. n=24
9 What is the relative humidity in the incubator?

The relative humidity in the incubator should be at 95-98% to minimize evaporation from the wells.

10 How often is the door of the incubator opened during an experiment?

If the incubator is shared by many different users requiring cell culture manipulations, make sure that the door is not opened too long to minimize environmental variations during the course of an experiment.

11 How many flasks, plates, etc. are in the incubator in addition to the RTCA Station?

When the interior of the incubator is filled with many other items in addition to the RTCA Station, proper air flow and convection could be disturbed, resulting in increased evaporation and uneven experimental conditions in the E-Plate 96.

12 Is there an additional tray of water at the bottom of the incubator to increase/stabilize humidity?

The relative humidity in the incubator should be at 95-98% to minimize evaporation inside the wells. If your type of incubator is rather slow in adjusting to this value (e.g., after opening the door), placement of an additional tray of water at the bottom of the incubator can significantly increase the humidity assuring its even distribution inside the incubator.

If edge well effects still persist after addressing the recommendations described above, also make sure to add water to the troughs between the wells in the E-Plate 96 to minimize evaporation. Always fill the troughs to approximately the same vertical height as the wells.
Is it possible that your incubator is negatively influencing the growth of the cells?

If you have a 2nd incubator, set up the following experiment: Prepare two E-Plates 96 in parallel (A & B). Place plate A into the RTCA Station in the 1st incubator and perform background reading, cell seeding and 1st measurement. Then put plate A into the 2nd incubator.

Similarly, start plate B in the 1st incubator on the RTCA Station generating a new experiment file. Leave plate B in the 1st incubator. Grow cells in both plates (A & B) to the desired density and check if differences appear in the edge wells (i.e., plate A in 2nd incubator vs. plate B in 1st incubator).

If you observe significant differences, turn off and clean the incubator causing edge well effects by wiping down interior surfaces with a towel moistened with 80% ethanol. Leave the door open until the ethanol has completely evaporated; then restart the incubator.

If this procedure identifies a problem with the incubator you are currently using, change to the better incubator and contact the service technician for the incubator for assistance.

For further assistance on how best to use RTCA Instruments, please contact ACEA Technical Support (techsupport@acebio.com) for assistance.

Trademarks:
xCELLigence, E-PLATE and ACEA BIOSCIENCES are registered trademarks of ACEA Biosciences - A part of Agilent in the US and other countries.

Intended Use:
For life science research only. Not for use in diagnostic procedures.