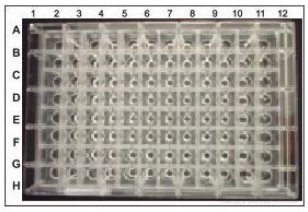
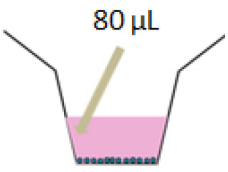
Seeding Adherent Cells in Agilent Seahorse XF96 Cell Culture Microplates

Basic Procedure



Agilent Seahorse XF Assays are performed in an Agilent Seahorse 96-well XF Cell Culture Microplate in conjunction with an XF^{e}96/XF96 Sensor Cartridge. Each microplate is formatted in a typical 96-well design, as shown. The seeding surface of each well is 0.106 cm^2, smaller than in a typical 96-well plate, but larger than in a typical 384 well plate. This procedure describes recommendations for seeding adherent cell types for use with the Agilent Seahorse XF^{e}96/XF96 Analyzer.

1. Harvest and re-suspend the cells to desired final concentration to seed in 80 µL of growth medium. Optimal cell seeding numbers vary widely, though are typically between 5,000 – 40,000 cells per well and must be determined empirically. For further information on optimal cell density, please see the Agilent Seahorse Cell Reference Database (http://www.agilent.com/cell-reference-database/) and/or XF Assay Guides and Templates http://www.agilent.com/en-us/support/cell-analysis-(seahorse)/seahorse-assay-guides-templates.
2. Seed 80 µL of cell suspension per well (as shown in figure below); do not seed cells in background correction wells (A1, A12, H1, H12). Be sure to put medium only (no cells) in the background correction wells.
3. Optional: Allow plate to rest at room temperature in the tissue culture hood for one hour. This can promote even cell distribution and reduce edge effects for some cell types[1]. Monitor adherence using a microscope.
4. Allow the cells to grow overnight in a cell culture incubator. Monitor growth and health of cells using a microscope.



Hint: Hold the pipette tip at an angle about halfway down the side of the wells for best technique and most homogeneous cell layer.

[1]. Lundholt BK, Scudder KM, Pagliaro L. A simple technique for reducing edge effect in cell-based assays. J Biomol Screen. 2003 Oct;8(5):566-7

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