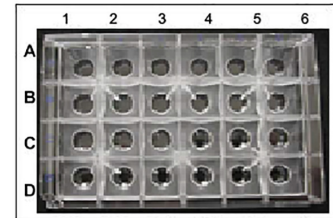




# Seeding Adherent Cells in Agilent Seahorse XF24 Cell Culture Microplates

## Basic Procedure

Agilent Seahorse XF Assays are performed in a 24-well XF Cell Culture Microplate in conjunction with an Agilent Seahorse XF<sup>e</sup>24/XF24 Sensor Cartridge. Each microplate is formatted in a typical 24-well design, as shown. The seeding surface of each well is similar to that of a typical 96-well (0.275 cm<sup>2</sup>).



This procedure describes recommendations for seeding adherent cell types for use with the XF<sup>e</sup>24/XF24 Analyzer.

A two-step seeding process is recommended when seeding Agilent Seahorse XF24 Cell Culture Microplates. The two-step process produces a consistent and even monolayer of cells.

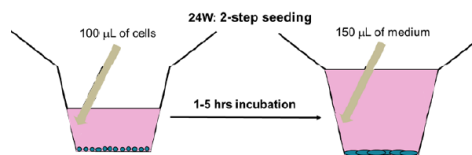
1. Harvest and re-suspend the cells to desired final concentration to seed in 100  $\mu$ L of growth medium. Optimal cell seeding numbers vary widely, though are typically between 10,000 – 80,000 cells per well and must be determined empirically. Agilent Seahorse Cell Reference Database (<http://www.agilent.com/cell-reference-database/>) and/or XF Assay Guides and Templates [http://www.agilent.com/en-us/support/cell-analysis-\(seahorse\)/seahorse-assay-guides-templates](http://www.agilent.com/en-us/support/cell-analysis-(seahorse)/seahorse-assay-guides-templates).
2. Seed 100  $\mu$ L of cell suspension per well (as shown in figure below); do not seed cells in background correction wells (A1, B4, C3, D6). Be sure to put medium only (no cells) in the background correction wells.  
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<sup>1</sup>.



- Place plate in a standard cell culture incubator to allow cells to adhere. This generally takes approximately 1 hour for strongly adherent cells, but may take 5-6 hours for less adherent cell types. Monitor adherence using a microscope.
- After cells have adhered, add 150  $\mu\text{L}$  of growth medium to each well (see figure below), bringing the total volume of medium in the well to 250  $\mu\text{L}$ . When adding medium to the wells, add it slowly to the sides as not to disturb the newly attached cells.
- 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.

<sup>1</sup>. 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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