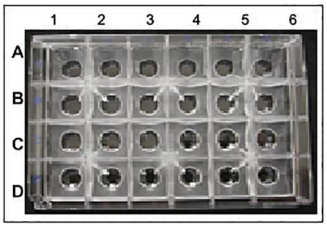
Seeding Adherent Cells in Agilent Seahorse XF24 Cell Culture Microplates

Basic Procedure



Agilent Seahorse XF Assays are performed in a 24-well XF Cell Culture Microplate in conjunction with an Agilent Seahorse XF^{e}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^{2}). This procedure describes recommendations for seeding adherent cell types for use with the XF^{e}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 µL 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.
2. Seed 100 µL 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[1].

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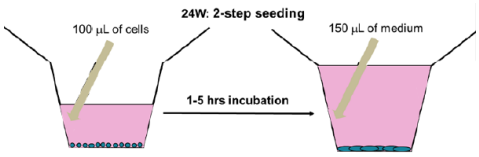
3. Place plate in a standard cell culture incubator to allow cells to adhere. This generally takes approximately 1 hour for strongly adherent cells, but may take 5-6 hours for less adherent cell types. Monitor adherence using a microscope.
4. After cells have adhered, add 150 µl of growth medium to each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