

Seeding Adherent Cells in Agilent Seahorse XFp Cell Culture Miniplates

Basic Procedure

Introduction

XF assays are performed in an Agilent Seahorse XFp Miniplate in conjunction with an Agilent Seahorse XFp Sensor Cartridge. Each miniplate is formatted as a single column of a typical 96-well plate. The seeding surface of each well is 0.106 cm^2 , approx. 40% the of the bottom surface area of a standard 96-well plate. This procedure describes recommendations for seeding adherent cells for use with the Agilent Seahorse XFp Analyzer.

Agilent Seahorse XFp Carrier Trays are included with each instrument and available separately. These carriers are designed to hold 3 XFp Miniplates and provide easier handling and manipulation of the plates while in the tissue culture hood or cell culture incubator. They are also compatible with microplate centrifuge adapters and most plate readers. The procedure given below can be performed with the XFp Miniplate inserted either in the carrier or on its own.



Procedure

1. Remove a three-pack of miniplates from the blue box. Remove the foil seal from the tub(s) that you will be using.
2. Add sterile water or PBS to the moat around the cell culture wells. Use an 8-channel pipettor set to 200 μL , and fill both sides of the moat (two tips will fit into each chamber). If no multi-channel pipette is available, fill each chamber of the moat with 400 μL of sterile water or PBS (total 3200 μL).
3. Add 80 μL of growth medium only (no cells) to wells A and H. These are background correction wells.
4. Determine the desired seeding concentration. Optimal cell seeding numbers can vary widely but are typically between 5×10^3 and 4×10^4 cells per well. Cells are seeded in 80 μL of growth media. For further information on optimal cell density, please see http://www.agilent.com/cs/library/usermanuals/public/New_Cell_Line_Characterization.pdf. Also see the Agilent Seahorse Cell Reference Database (<http://www.agilent.com/cell-reference-database/>) and/or Seahorse 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)).
5. Harvest the cells using standard procedures. Resuspend the cells in appropriate growth medium, count, and then dilute to the desired seeding concentration.
Example: After harvesting and counting, the cell concentration is 1.6×10^6 cells per mL. To achieve the desired seeding concentration, the dilution factor is 1.6×10^6 cells per mL / 2.5×10^5 cells per mL = 6.4.
For one XFp plate: Combine 100 μL of cells with 540 μL of growth medium.
6. Add 80 μL of the cell suspension to wells B-G (as shown in the figure).
7. Allow the cells to grow overnight in a cell culture incubator. For cells being cultured for longer periods, ensure that the moat does not dry out. Replenish fluids when a medium exchange is performed on the cells.
8. Check the growth and health of cells using a microscope.
NOTE: Do NOT add fluid to the moat prior to running the assay. It is not necessary to remove fluid from the moat chambers prior to the run.

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

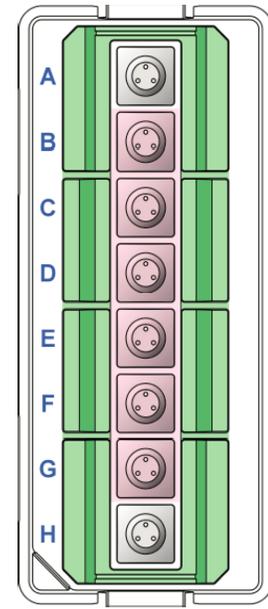
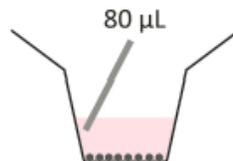


Diagram of Seahorse XFp Miniplate highlighting moat with 8 chambers (green) and 6 assay wells (pink). The background wells are not colored.

Learn more

www.agilent.com/en-us/promotions/seahorse-xf-technology

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