Seeding Adherent Cells in Seahorse XFp Cell Culture Miniplates

Introduction

XF assays are performed in a Seahorse XFp Miniplate in conjunction with an XFp Sensor Cartridge. Each miniplate is formatted as a column of a typical 96-well plate. The seeding surface of each will 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 Seahorse XFp Analyzer.

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, fold it back and forth along the perforations a few times then pull the tubs apart. 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. Consult the literature[1] and/or test different cell seeding densities to ensure good results. Optimal cell seeding numbers can vary widely but are typically between 5 x 10^3 and 4 x 10^4 cells per well. Cells are seeded in 80 µL of growth media.

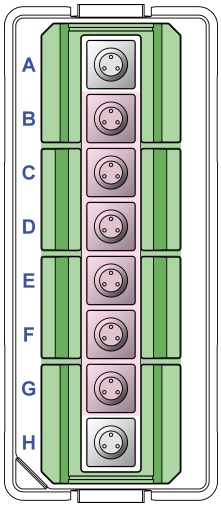

Diagram of Seahorse XFp Miniplate highlighting moat with 8 chambers (green) and 6 assay wells (pink). The background wells are not colored.

[1] *Cell Line Reference Database:* http://www.seahorsebio.com/learning/cell-line.php

Example: One wants to seed at 2.0 x 10^4 cells per well. Thus, the target cell seeding concentrations is 2.0 x 10^4 cells / 0.080 mL = 2.5 x 10^5 cells per mL.

5. Harvest the cells using standard procedures. Resuspend the cells in growth medium, count them, and then dilute to the desired seeding concentration. See example below.

Example: Following harvesting and counting, the cell concentration is 1.6 x 10^6 cells per mL. To achieve the desired seeding concentration, the dilution factor is 1.6 x 10^6 cells per mL/2.5 x 10^5 cells per mL = 6.4. Combine 100 µL of cells with 540 µL of growth medium (or other proper ration of volumes).

6. Add 80 µL of 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, make sure that the moat does not dry out. Replenish fluids whenever you do a medium exchange on the cells.
8. Check the growth and health of cells using a microscope.
   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.