Seeding Suspension in Seahorse XFp Cell Culture Miniplates

Introduction

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

Because the measurement of oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) takes place in the microchamber formed at the bottom of the specialized Seahorse XFp Miniplate, suspension cells should be adhered to the bottom of the plate. Seahorse XFp Miniplates can be centrifuged in a standard microplate centrifuge using the Seahorse XFp Miniplate Carriers (see next page).

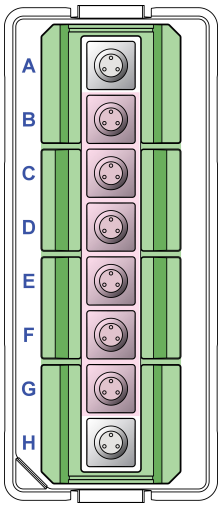

Diagram of Seahorse XFp Miniplate highlighting moat with 8 chambers (green) and 6 assay wells (pink). The background wells are not colored.

Procedure

1. Remove a three-pack of miniplates from the blue box, fold it back and forth along one of the perforations a few times then pull the tubs apart. Remove the foil seal from the tub(s) that you will be using.
2. Some cell lines require a plate coating in order to adhere. Plate coating examples include Cell-Tak, poly-D-lysine, and gelatin. The plate coating will depend on the specific cell type being used in the assay.
3. Determine the optimal seeding concentration. Consult the literature[1] and/or test different cell seeding densities to ensure good results. For suspension cells, the optimal seeding density is typically between 1 x 10^5 and 4 x 10^5 cells per well.[2]
4. Add 50 µL assay medium (no cells) to wells A and H. These are Background Correction wells.
5. Harvest and dilute the cells in assay medium[3] to the desired concentration, where:
   desired # cells/well x 20 = # cells/mL

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

[2] *Note that these values are larger than for adherent cells because (1) suspension cells are often much smaller than adherent cells and (2) they usually have significantly lower rates of basal or resting respiration.*

[3] *The choice of assay medium depends on the type of experiment being performed. Please see the basic procedure "preparation of Assay Media" for further details.*

6. Add 50 µL of cell suspension to wells B through G; do not add cells to the Background Correction wells (wells A and H).
7. Place the miniplate(s) in a carrier tray and centrifuge at 300 x g for 1 min with no brake. Seahorse XFp Miniplate Carriers are included with each instrument when purchased, and also available separately. These carriers are designed to hold up to 3 miniplates, and fit standard microplate centrifuge adapters. Ensure that the centrifuge rotor is balanced appropriately.

8. Use microscope to visually confirm adherence of the cells to the well bottom.
9. Taking care not to disturb the cells on the bottom, gently add assay medium to each well to the desired initial assay volume (usually 180 µL). *More details on volumes for use with Seahorse XF Assay Kits can be found in the Seahorse XF Stress Test User Guides.*
10. Add sterile water or PBS to the moat around the cell culture wells, 100 µL per chamber[4]. Using an 8-channel pipettor (if available) set to 50 µL, fill both sides of the moat using two tips per chamber. If no multi-channel pipette is available, individually fill each chamber of the moat with 100 µL of sterile water or PBS (total 800 µL).
11. Place the Seahorse XFp Miniplate(s) in a carrier tray in a 37°C non-CO_2 incubator for 30 minutes to equilibrate the temperature.

[4] *For cells that are plated the same day as the assay is run, just a small amount of fluid in the moat is recommended. For cells being incubated overnight prior to assay (such as adherent cells) it is necessary to fill the moat with 400 µL per chamber to avoid assay well evaporation and edge effects.*