Seeding Suspension Cells in Agilent Seahorse XFp Cell Culture Miniplates

Basic Procedure

Introduction

XF assays are performed in a 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, as shown below. 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 Agilent 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 XFp Miniplate, suspension cells must be adhered to the bottom of the wells.

XFp Miniplates can be centrifuged in a standard centrifuge with microplate adapters using the Agilent Seahorse XFp Miniplate Carriers.

Procedure

1. Remove a three-pack of miniplates from the blue box. Remove the foil seal from the tub(s) that will be used.
2. Some cell types require a plate coating for adherence. 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.

Agilent Technologies

3. Determine the optimal seeding concentration. For further information on optimal cell density, please 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). For suspension cells, the optimal seeding density is typically between 1 x 10^5 and 4 x 10^5 cells per well.
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 appropriate assay medium to the desired concentration where: desired # cells/well x 20 = # cells/mL. For choosing and preparing the appropriate assay medium, please see http://www.agilent.com/cs/library/selectionguide/public/5991-7878EN.pdf and http://www.agilent.com/cs/library/usermanuals/public/Media%20 Prep %20XFp.pdf.
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. These carriers are designed to hold up to 3 miniplates, and fit standard centrifuge microplate adapters. Ensure that the centrifuge rotor is balanced appropriately.
8. After centrifugation, 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).
10. Add sterile water or PBS to the moat around the cell culture wells, 100 µL per chamber[2]. 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 XFp Miniplate(s) in a carrier tray in a non-CO_2 incubator at 37˚C for 30 minutes to equilibrate temperature.

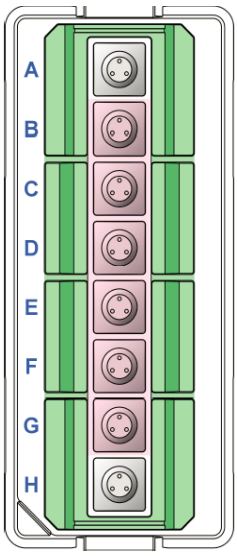

Diagram of Seahorse XFp Miniplate highlighting moat with 8 chambers (green) and 6 assay wells (pink). The background correction wells are not colored.

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

[2]. For cells that are plated the same day as the assay is run, a smaller 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.

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

For Research Use Only. Not for use in diagnostic procedures.

Product specifications and descriptions in this document are subject to change without notice.

Published in the USA, June 30, 2017

Agilent Technologies