Protocol

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Seeding Adherent Cells in Agilent Seahorse XF Pro M Cell Culture Microplates

Introduction

Agilent Seahorse XF assays are performed in an Agilent Seahorse XF Pro M cell culture microplate with the Agilent Seahorse XFe96/XF Pro sensor cartridge. Each microplate is formatted in a typical 96-well and 4-moat well design. This procedure describes the recommendations for seeding adherent cells for best results when performing Seahorse XF assays using the Agilent Seahorse XF Pro analyzer.

Basic cell seeding workflow

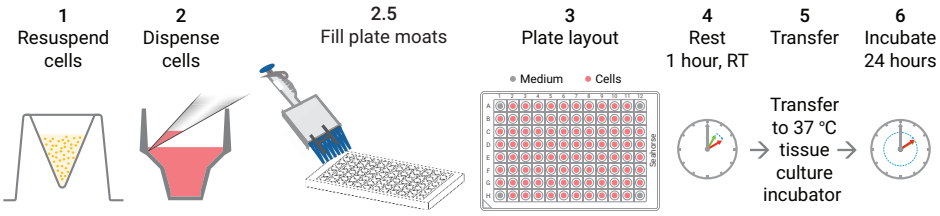

Figure 1. Agilent Seahorse XF tissue culture microplate cell seeding procedure workflow.

1. Harvest and resuspend the cells to the desired final concentration for seeding in 80 µL of growth medium. Please refer to the chart below for preparation of common cell seeding densities. Either: 1) dilute the cell suspension to 1 × 10^6 cells per mL (cells/mL) and dilute again to the desired density based on the table below, or 2) dilute the cell suspension to the desired cell density using Equation 1, where 'cells' is the number of cells, and 'volume' is in mL.

Equation 1: $cells^{stock} \times volume^{stock} = cells^{final} \times volume^{final}$

2. Seed 80 µL of cell suspension per well (cells/well), as shown in Figure 1. Do not seed cells in background correction wells (A1, A12, H1, H12).
3. Add medium only (no cells) to the background correction wells.

4. Add 1.0 mL of cell culture-grade water to each of the moat wells (four of them in total). This can be done by setting an 8-channel pipette to 250 µL and pipetting into two sections at a time. Dispense in the moat wells parallel to columns 1 and 12 to fill all four sections, as shown in Figure 2.

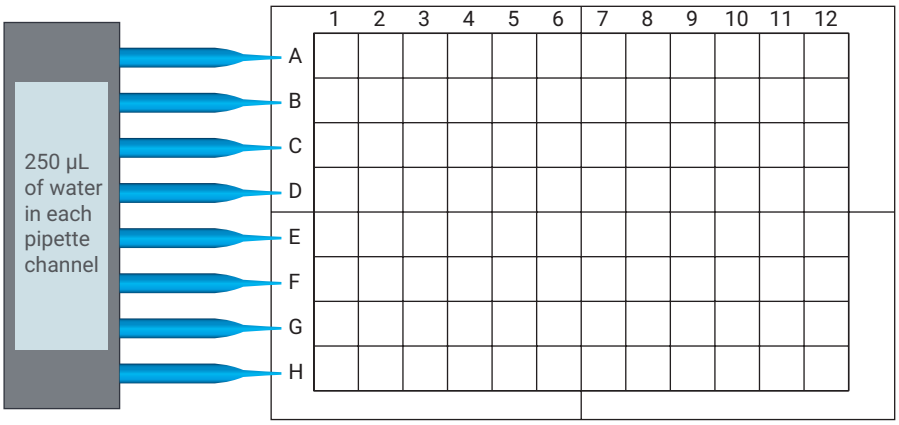

Figure 2. Filling the moat wells using an 8-channel pipette.

5. **Important:** Allow plate to rest at room temperature in the tissue culture hood for one hour.
6. After the one hour rest, transfer the plate with cells to a properly humidified 37 °C CO_2 incubator. Please do not tilt or shake the plate during transfer, to avoid water spilling out of the moat wells.
7. Allow the cells to grow overnight in a tissue culture incubator. Monitor the growth and health of cells using a microscope.

Detailed procedure

Choosing a seeding density

Optimal cell seeding numbers vary widely based on cell type, but are typically between 0.5 to 4.0 × 10^4 cells per well and must be determined empirically.

Example: One desires to seed one XF Pro M microplate at 2.0 × 10^4 cells/well, and a stock solution of 4.20 × 10^6 cells/mL is obtained. Since one plate is needed, a total volume of 10 mL diluted cell suspension at a density of 2.5 × 10^5 cells/mL is required (i.e., 2.0 × 10^4 cells/well ÷ 0.08 mL/well = 2.5 × 10^5 cells/well).

Using Equation 1: (4.20 × 10^6 cells) × (X mL) = (2.5 × 10^5 cells) × (10 mL)

Solving for X, one obtains 0.595 mL. Therefore, add 0.595 mL of stock cell suspension to 9.405 mL of appropriate cell culture media to obtain 10 mL of 2.5 × 10^5 cells/mL, which provides a final cell density of 2.0 × 10^4 cells/well in 80 µL of cell culture media.

For further information on optimal cell density, please see the Agilent Seahorse XF Assay Learning Center including Related Support and Reference Materials: Cell Reference Database.

Harvesting and resuspending cells

1. A single cell suspension is optimal for producing a consistent cell monolayer when cells are seeded. It is beneficial to break up any aggregated cells prior to seeding. It is also important to ensure that cells are thoroughly resuspended before counting and seeding into XF Pro M cell culture microplates. For detailed information regarding best practices for cell culture and seeding cells in XF cell culture microplates, see Knowing your Cells of Interest: Advice and Suggestions for a Successful XF Experience (PDF).

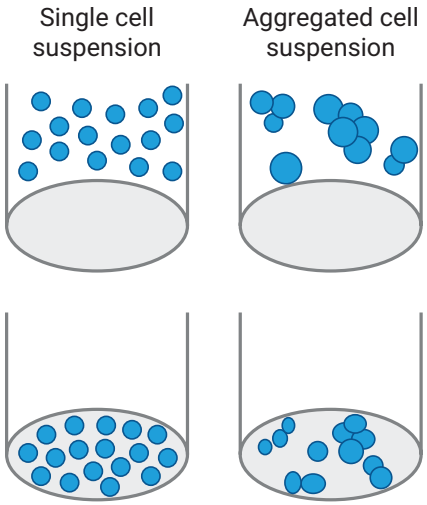

Figure 3. Single cell versus aggregated cell suspension, and resulting distribution of cells within the well.

Table 1. Agilent Seahorse XF Pro M microplate cell seeding dilution table.

Desired Cell Density Per Well (Number of Cells /80 µL Media)	Stock Cell Suspension (mL) (1×10^6 Cells/mL)	Growth Media (mL)	Minimal Volume for One XF Pro M Microplate (mL)
0.5×10^4	0.5	7.5	8.0
1.0×10^4	1.0	7.0	8.0
1.5×10^4	1.5	6.5	8.0
2.0×10^4	2.0	6.0	8.0
2.5×10^4	2.5	5.5	8.0
3.0×10^4	3.0	5.0	8.0
4.0×10^4	4.0	4.0	8.0

2. When diluting cells to the desired seeding density, mix the full volume two to three times via pipette to ensure a homogeneous suspension.

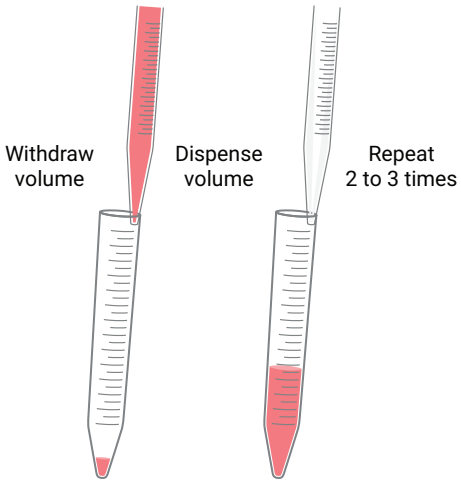

Figure 4. Dilute and mix cells thoroughly.

3. Transfer the diluted and mixed cells to a pipetting reservoir.
4. Cells may subsequently begin to settle in the reservoir after mixing, especially for larger cell types. Therefore, it is recommended to gently mix the remaining cells in the reservoir during the seeding procedure; usually one or two additional mixes via pipette are sufficient when seeding a single plate. If the same suspension of cells is being used to seed multiple plates, the cell suspension should also be remixed between seeding each plate.

Dispensing the cells into the XF cell culture microplate

1. When dispensing cells into the XF Pro M cell culture microplate, an 8- or 12-channel, 20 to 200 µL multipipette is recommended for convenience, speed, and consistency among wells and/or plates.
2. The pipette tips should be at ~45 °, approximately halfway down the side wall, and should touch the wall of the well (Figure 5). The pipette should be held at the same angle for all wells.
   A. The tips should be slightly submerged in the media after the cells are dispensed.
   B. An electronic pipet may improve the consistency of dispensing volume.

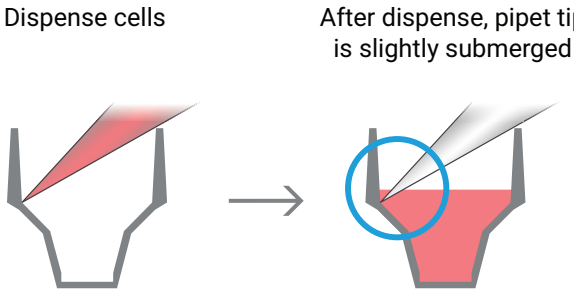

Figure 5. Proper pipetting technique for dispensing cells into the Agilent Seahorse XF tissue culture microplates.

Rest and incubation of the cells

1. Allow the cells to rest at room temperature in the cell culture hood for one hour after seeding. Note that cell plates may be gently moved to the rear portion of the biosafety cabinet for the rest step if desired. A rapid change in temperature immediately after cell seeding can cause significant edge growth effects in an XF Pro M cell culture microplate.
2. Place cells in the tissue culture incubator.
   A. The incubator should be properly humidified to prevent excess evaporation from the wells. Ensure the water pan in the tissue culture incubator is at the recommended volume.
   B. Place XF Pro cell culture microplates toward the back of the incubator to minimize exposure to temperature and humidity changes when the incubator is accessed.
   C. If resources allow, an incubator used only for the incubation of assay plates may help reduce the impact of frequent incubator door opening and closing.

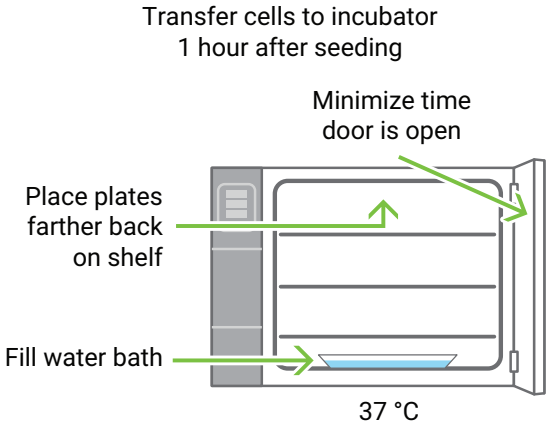

Figure 6. Transfer cells to a humidified incubator.

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