Basic Procedure

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Seeding Adherent Cells in Agilent Seahorse XF HS Miniplates

This procedure is intended for use with XF HS Miniplates or XF HS PDL Miniplates.

Introduction

XF assays are performed in Agilent Seahorse Cell Culture Plates in conjunction with Agilent Seahorse XFp Sensor Cartridges. The purpose of the XF HS Miniplate design is to allow performance of XF assays with significantly fewer cells per well, facilitating functional analysis of non-proliferative or limited amounts of cells. The seeding area of the well is 0.031 cm^2, approximately 30% of the area of standard XFp (or XF96) cell culture plates.

Note: Optimization of cell density is recommended when initially using the XF HS Tissue Culture Miniplate.

Each XF HS Miniplate consists of an 8-well Cell Culture Plate with a raised "ring" element in the center of each well (Figure 1A). The XF HS Miniplates are tissue culture treated and gamma irradiated. Each XF HS Miniplate is pre-assembled with a silicone cell-seeding mask and plate lid. Each package also includes a mask removal tool (Figure 1B).

This document describes the process for seeding adherent cells in Agilent XF HS Miniplates.

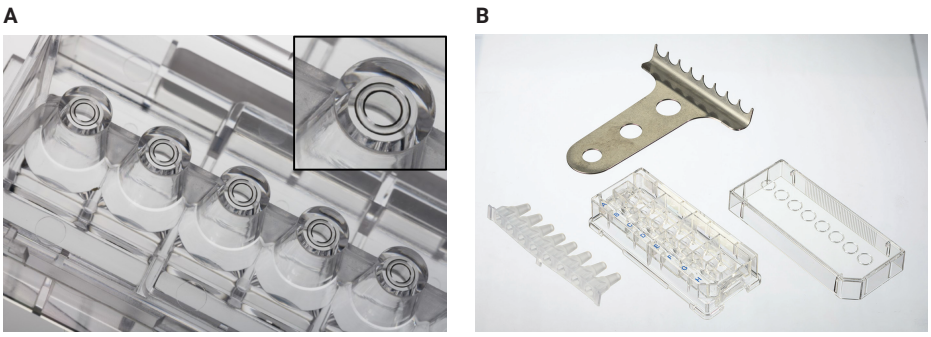

Figure 1. (A) The ring element in the wells of the XF HS Miniplate encircle the reduced cell-seeding area. (B) Each plate is pre-assembled with a silicone cell-seeding mask, XF HS Miniplate Lid, and includes a reusable mask removal tool.

Procedure

Day prior to assay

Seeding cells

1. Obtain the XF HS Miniplates and remove the foil seal(s) from the plate(s) that will be used.
2. Add sterile water or PBS to the moat around the cell culture wells (Figure 2).
    a. Use an 8-channel pipettor set to 200 µL, and fill both sides of the moat (two tips will fit into each chamber).
    b. If no multichannel pipette is available, fill each chamber of the moat with 400 µL sterile water or PBS (total 3,200 µL).

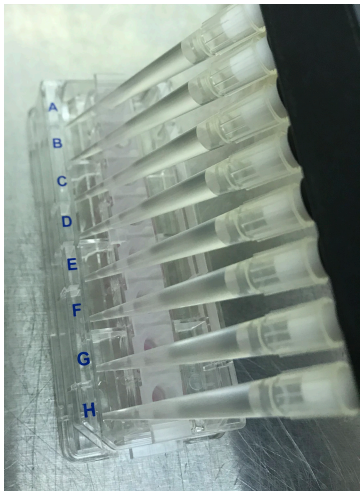

Figure 2. An 8-channel pipettor is used to load the plate moats on either side of the row of wells.

3. Determine the desired seeding concentration. Optimal cell seeding numbers can vary widely but are typically between 1.0×10^3 and 1.0×10^4 cells per well. Refer to the Agilent Cell Analysis Publication Database to search for cell seeding density recommendations by cell type. The XF HS Miniplate will usually accommodate one-third the number of cells that an XF96 or XFp Cell Culture plate requires.
4. 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 7.5×10^5 cells/mL. To achieve the desired seeding concentration, the dilution factor is 7.5×10^5 cells/mL / 1.0×10^5 cells/mL = 7.5. For one XF HS Miniplate, combine 100 µL of cells with 650 µL of growth medium.

5. Add 30 µL of the cell suspension to the inner ring element of wells B through G. Depress the plunger gently but consistently to dispense the cell suspension in a single XF HS Miniplate well.
    a. 20 to 200 µL (or smaller) pipette tips are recommended for use with the silicone mask.
    b. It is recommended to seed cells one well at a time in XF HS Miniplates – the pipette tip must be placed at the bottom of the well to dispense properly (Figure 3).

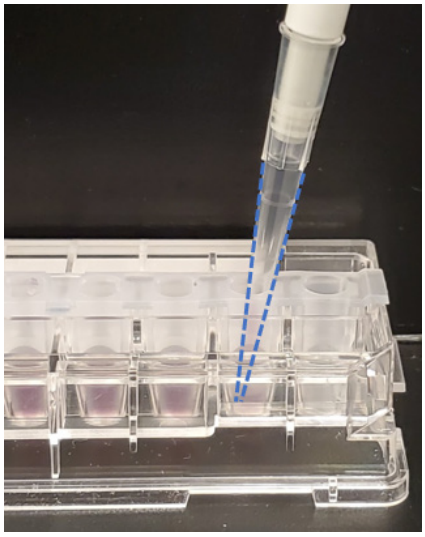

Figure 3. Pipette tip (outlined in blue) is inserted to the bottom of each well to dispense cell suspension.

6. Add 30 µL growth media (without cells) to wells A and H – these are background correction wells.

Notes: In the event that bubbles form at the bottom of the well (Figure 4), the plates may be centrifuged 1 to 2 minutes at 200 × g to remove any bubbles present.

A **B**

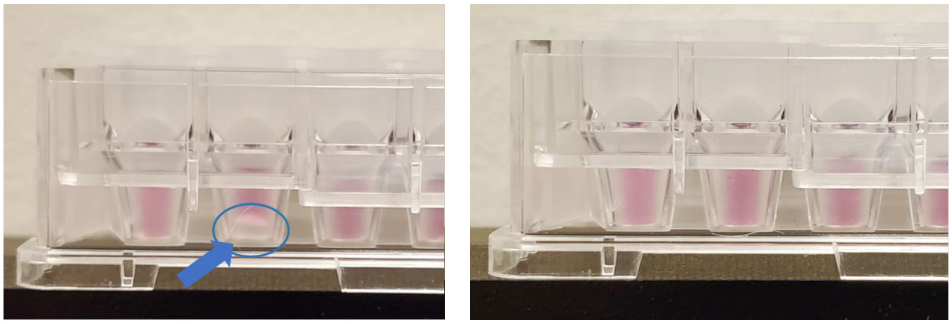

Figure 4. (A) (Before) A bubble is present at the bottom of the notated well, (B) (After)The bubble was released during centrifugation.

IMPORTANT: Allow plate to rest at room temperature in the tissue culture hood for one hour. This can promote even cell distribution for certain cell types.[1,2]

7. Monitor adherence using a microscope.

8. After one hour rest at ambient temperature, allow the cells to grow overnight in a cell culture incubator.

Note: The XF HS Miniplates are compatible with the Agilent XFp Miniplate Carrier Trays (part number 103057-100).

Prepare XFp Sensor Cartridge

Refer to the Basic procedures for hydrating the XFp cartridge in the XF Learning Center for additional information

1. Aliquot at least 5 mL of XF Calibrant into a 15 mL conical tube.
2. Place the conical tube containing XF Calibrant in a non-CO_2 37 °C incubator overnight.
3. Remove a three-pack of cartridges from the green box. Remove the foil seal from the tub(s) that will be used.
4. Separate the utility plate and Agilent Seahorse Sensor Cartridge. Place the sensor cartridge upside down on the lab bench.
5. Fill each well of the utility plate with 200 µL of sterile water.
6. Fill the moats around the outside of the wells with 400 µL of sterile water per chamber (Figure 5).
7. Return the XFp Sensor Cartridge to the utility plate with sterile water.
8. Place the cartridge/utility plate assembly in a non-CO_2 37 °C incubator overnight.

Figure 5. An 8-channel P200 pipettor is used to fill the cartridge moats.

Design experiment

Go to the "Design Experiment" section of the XF Learning Center for instruction on preparing a template for XF Analysis.

Day of assay

Prepare XFp Sensor Cartridge:

Refer to the Prepare the Cartridge section of the XF Learning Center for more information

1. Remove the Sensor Cartridge assembled with utility plate and prewarmed XF Calibrant from the incubator.
2. Lift the Sensor Cartridge off the utility plate, and place upside down next to the utility plate.
3. Remove and discard the water from the utility plate.
4. Fill each well of the utility plate with 200 µL of the prewarmed XF Calibrant.
5. Fill the moats around the outside of the wells with 400 µL of XF Calibrant per chamber.
6. Lower the sensor cartridge onto the utility plate, submerging the sensors in calibrant.
7. Place the assembled sensor cartridge with utility plate in a non-CO_2 37°C incubator for at least 60 minutes prior to loading the injection ports for the assay.

Prepare XF Assay Medium:

Refer to the appropriate XF Kit User Guide or the XF Learning Center for "Preparing XF Assay Medium".

Prepare Cell Plate for XF Analysis:

Remove the mask using the mask removal tool:

a. With one hand, hold the plate flat on the bench or working surface. With the other hand, insert the tool between the top of the plate and the mask.
b. The prongs of the removal tool should remain parallel to the top surface of the plate while they're being inserted.
c. Do not lever them back and forth to insert the tool further – this will create suction and potentially disturb the cell layer.
d. The goal is to remove the mask in one motion from all wells at the same time.
e. Once the tool has been inserted completely, then use it as a lever to remove the mask.
f. The mask will not stay on the prongs securely – once the mask begins to separate from the plate, use a finger to hold it onto the tool so it does not fall onto the plate.
g. Once removed, dispose of the plate mask.

Figure 6. (A) insert the prongs of the removal tool between each well, keeping the bottom of the tool parallel to the top of the plate. (B) once the prongs are inserted, lever the mask from the wells of the plate. (C) Hold the mask onto the removal tool with one finger as its being lifted from the plate to prevent it from falling.

Note: Media may be removed with the removal of the silicone mask. Approximately 20 µL of medium will be left in each well. If inconsistent volumes remain, carefully remove media only from the outer-ring area of the well, taking care not to touch the cells within the ring. Always leave some media to cover the cells. Small differences in the overall volume of each well will not impact OCR or ECAR, however the final concentration of injected reagents may be affected.

Wash cells with XF Assay Medium:

1. Ensure cells are adhered, with a consistent monolayer.
2. Make sure there are no cells in the background correction wells.
3. Gently add 200 µL of assay medium to each well.
4. Remove all but 20 µL of the assay medium from each well.
5. Repeat the wash process – replace 200 µL of media and remove all but 20 µL.
6. Finally, add back assay medium (~160 µL) to reach a starting volume of 180 µL.
7. Observe the cell layer after washing. Place the washed cell plate in a 37 °C non-CO_2 incubator for 60 minutes prior to performing the assay.

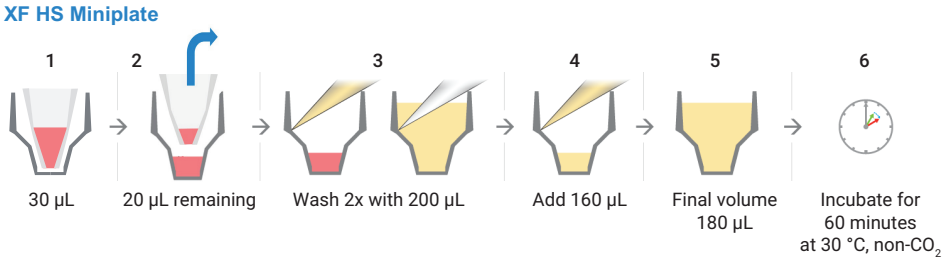

Figure 7. Schematic illustration displays the steps required for removing the silicone mask (step 2) and washing samples in XF HS Miniplates with XF Assay Medium.

Assemble injection solutions

Refer to the appropriate XF Kit User Guide or Refer to the XF Learning Center section "Assemble Injection Solutions" for instructions to prepare injection solutions and load the XFp Sensor Cartridge as required and defined by the assay protocol.

Run the XF Assay

Go to the "Run Assay" Section of the XF Learning Center for instructions on final steps for performing an XF HS Mini Assay.

Note: Upon loading the XF HS Miniplate into the XF HS Mini Analyzer, the screen will present a reminder to remove the silicone mask (Figure 8). This must be done to prevent interference and possible damage to the instrument

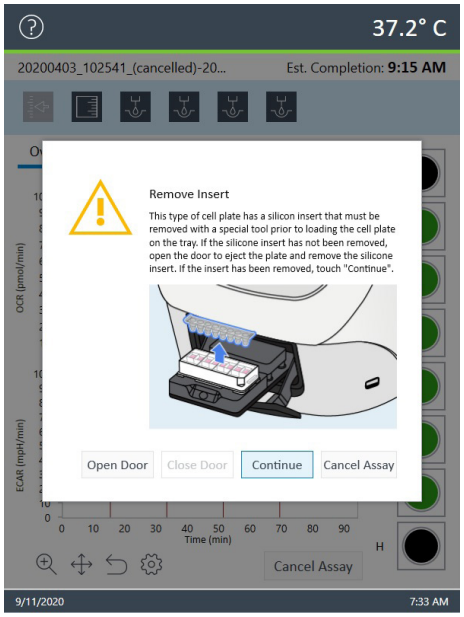

Figure 8. The XF HS Mini Analyzer prompts the user to remove the cell-seeding mask.

References

1. Methods for Reducing Cell Growth Edge Effects in Agilent Seahorse XF Cell Culture Microplates, *Agilent Technologies*, publication number 5994-0240EN, **2019**.
2. Lundholt, B. K.; Scudder, K. M.; Pagliaro, L. A Simple Technique for Reducing Edge Effect in Cell-Based Assays. *J. Biomol. Screen.* **2003** Oct, *8(5)*, 566–7.

www.agilent.com/chem/hsmini

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