Optional Procedure for Hydrating an Agilent Seahorse XFe96 Sensor Cartridge
For use with Agilent Seahorse XFe and XF96 Analyzers

The Basic Procedure for hydrating a sensor cartridge may not always eliminate bubbles that may form during the overnight incubation. Bubbles can cause negative oxygen consumption rates (OCRs) by interfering with the instrument calibration. If the XF data contains negative OCR data when using the Basic Procedure, we recommend the following procedure for subsequent assays.

Materials
Agilent Seahorse XFe96 FluxPaks contain:

• Agilent Seahorse XFe96 Extracellular Flux Assay Kits:
  • Cartridge Lid
  • Sensor Cartridge
  • Utility Plate
• Agilent Seahorse XF96 Cell Culture Microplates
• Agilent Seahorse XF Calibrant (500 mL)

Also required, but not included:

• 200-µL pipettor and tips
• 50-mL conical tubes
• Cell culture grade sterile water

Procedure
Day prior to assay
1. Aliquot at least 20 mL of XF Calibrant into a 50-mL conical tube.
2. Place this XF Calibrant in a non-CO₂ 37 °C incubator overnight.
3. Open the XFe96 Extracellular Flux Assay Kit, and remove the contents.
4. Place the sensor cartridge upside down next to the utility plate.
5. Fill each well of the utility plate with 200 µL of sterile water.
6. Lower the sensor cartridge onto the utility plate submerging the sensors in water.
7. Verify that the water level is high enough to keep the sensors submerged.
8. Place the assembled sensor cartridge and utility plate in a non-CO₂ 37 °C incubator overnight.
9. To prevent evaporation, verify that the incubator is properly humidified.
Day of assay

1. Remove the conical tube of calibrant and assembled sensor cartridge with the utility plate from the incubator.

2. Place the sensor cartridge upside down next to the utility plate.

3. Remove and discard water from the utility plate.

4. Fill each well of the utility plate with 200 µL of prewarmed XF Calibrant.

5. Lower the sensor cartridge onto the utility plate, submerging the sensors in calibrant.

6. Place the assembled sensor cartridge with the utility plate in a non-CO₂, 37 °C incubator for 45–60 minutes prior to loading the drug ports of the sensor cartridge.