User Guide

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How to Use the Agilent Seahorse XF Hydrobooster to Hydrate an Agilent Seahorse XFe96/XF Pro Sensor Cartridge

For use with the Agilent Seahorse XFe96 and XF Pro analyzers

An important component of the XF assay platform is the sensor cartridge. Each probe tip of the sensor cartridge is spotted with a solid-state sensor material, which detects changes in both pH and O_2 concentration over time to calculate rates. For the sensors to function correctly, they must be thoroughly hydrated.

This document describes the method of preparing a sensor cartridge before an XF assay using the Agilent Seahorse XF Hydrobooster, improving hydration and data quality. This method is designed to prevent bubble formation under the sensors during hydration, which can otherwise impact XF data quality and accuracy.

Important notes

- This procedure must be performed one day prior to using the sensor cartridge for an XF assay.
- The XF Hydrobooster must be removed prior to loading drug ports of the sensor cartridge.
- The XF Hydrobooster cannot be placed into the analyzers.

Failure to follow these notes may cause an inaccurate assay result.

Materials

Agilent Seahorse XFe96/XF Pro and XF Pro M FluxPaks, composed of:

1. Agilent Seahorse XFe96/XF Pro Extracellular Flux assay kit, containing:
   - Cartridge lid
   - Sensor cartridge
   - Hydrobooster
   - Utility plate

2. Agilent Seahorse XFe96/XF Pro or XF Pro M cell culture microplates
3. Agilent Seahorse XF calibrant (500 mL)

Also required, but not included

- 200 µL pipettor and tips
- 50 mL conical tubes
- NonCO_2 incubator at 37 °C

Procedure

Procedure day, one day before the assay

1. Open the Seahorse XFe96/XF Pro Extracellular Flux assay kit and remove the contents.
2. Place the sensor cartridge, upside down, next to the utility plate.
3. Fill each well of the utility plate with 200 µL of XF calibrant.
4. Place the XF Hydrobooster on top of the utility plate, and push the XF Hydrobooster downward to make sure it has tightly assembled with the utility plate.
5. Lower the sensor cartridge through the openings on the XF Hydrobooster plate into the utility plate, submerging the sensors in XF calibrant (Figure 1).

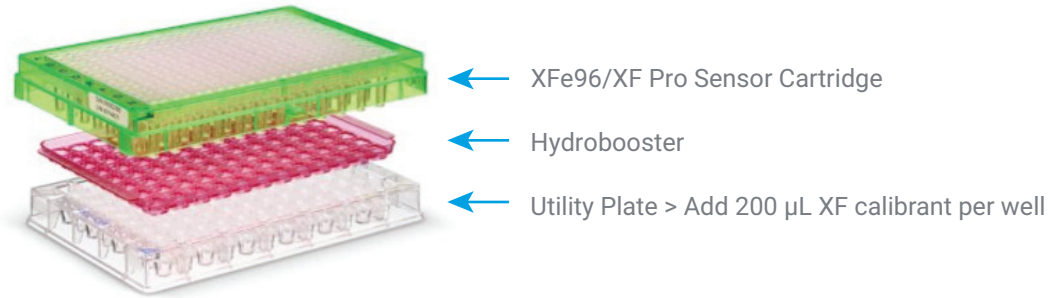

Figure 1. Assembly of the Agilent Seahorse XF Pro Hydrobooster with the Agilent Seahorse XFe96/XF Pro sensor cartridge and utility plate.

6. Verify that the XF calibrant level is high enough to keep the sensors submerged.
7. Place in a nonCO_2 incubator at 37 °C and leave overnight. To prevent evaporation of the XF calibrant, the incubator should be humidified.

Day of the assay

1. Remove the assembled sensor cartridge with the XF Hydrobooster and utility plate from the incubator.
2. Place the sensor cartridge, upside down, next to the utility plate.
3. Hold the utility plate steadily with one hand, and use the other hand to remove the XF Hydrobooster by lifting it from one corner.
4. Place the sensor cartridge back onto the utility plate, and load compounds into the ports as required. Place into the XFe96 or XF Pro analyzer for calibration.

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