

How to Hydrate a Seahorse XFp Sensor Cartridge

For use with the Seahorse XFp Analyzer

Introduction

A critical 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 that detects changes in both pH and O₂ concentration over time to calculate rates. In order for the sensors to function correctly, they must be thoroughly hydrated. The following procedure is designed to hydrate and prepare the sensor cartridges for the assay.

Materials

Seahorse XFp FluxPak containing:

1. Green Box:
 - a. Sensor cartridge (12x)
 - b. Utility plate (12x)
 - c. Cartridge lid (12x)
2. Blue Box:
 - a. Seahorse XFp Miniplate with lid (12x)
3. Seahorse XF Calibrant (100 mL)

Additional requirements: non-CO₂ incubator

Procedure

Day Prior to Assay:

1. Remove a three-pack of cartridges from the green box, fold it back and forth along the perforation a few times then pull the tubs apart. Remove the foil seal from the tub(s) that will be used.
2. Separate the utility plate and Sensor Cartridge (Figure 1). Place the sensor cartridge upside down on the lab bench (Figure 2).

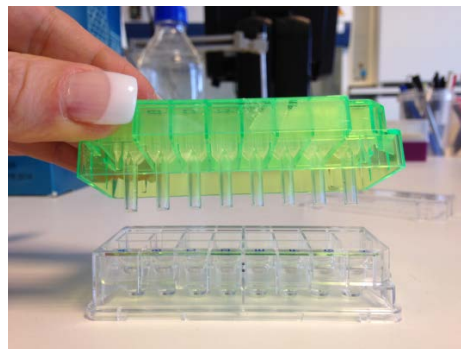


Figure 1 | Sensor cartridge being lifted from utility plate.

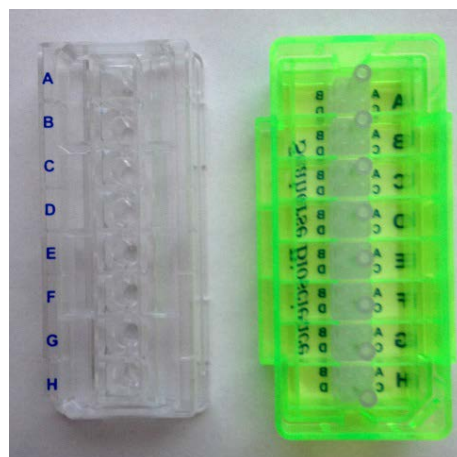


Figure 2 | Utility plate (left) next to upside down sensor cartridge (right).

- Fill each well of the utility plate with 200 μL of Seahorse XF Calibrant. See table below.
- Fill the moats around the outside of the wells with 400 μL per chamber. See table below.

Plate Compartment	single channel pipette setting	8-channel pipette setting
Wells	200 μL , 8 dispenses	200 μL , 1 dispense
Moat chambers	400 μL , 8 dispenses	200 μL , 2 tips per chamber, 2 dispenses

- Return the Seahorse XFp Sensor Cartridge to the utility plate that now contains calibrant.
- Place the cartridge assembly in a non- CO_2 37°C incubator overnight. To prevent evaporation of the Seahorse XF Calibrant, the incubator should be humidified.

Day of Assay:

- Following the overnight incubation, remove the cartridge assembly from the incubator.
- Lift the sensor cartridge completely out of the calibrant and utility plate.
- Immediately return the sensor cartridge back onto the utility plate, submerging the sensors in calibrant. This step eliminates any bubbles that may form during the overnight hydration.

NOTICE

New steps required for accurate calibration.

Seahorse XFp Carrier Trays are included with each instrument. These carriers can hold two (2) Seahorse XFp cartridge/miniplate assemblies or three (3) miniplates without cartridges. They provide easier handling and incubation of plates and cartridges as well as compatibility with microplate-based equipment.



Figure 3 | Seahorse XFp Carrier Tray holding 2 cartridges supported by miniplates.

Optional Procedure for Hydrating a Seahorse XFp Sensor Cartridge

For use with Seahorse XFp Analyzers

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

Materials

Seahorse XFp FluxPaks containing:

- Seahorse XFp Extracellular Flux Assay Kit
 - Cartridge Lid
 - Sensor Cartridge
 - Utility Plate
- Seahorse XFp Cell Culture Miniplates
- Seahorse XF Calibrant (100 mL)

Also required, but not included:
- 200 μ L pipettor and tips
- 15mL conical tubes
- Cell culture grade sterile water

Procedure

Day Prior to Assay

1. Aliquot at least 5 mL of Seahorse XF Calibrant into a 15 mL conical tube.
2. Place this Seahorse XF Calibrant in a non-CO₂ 37°C incubator overnight.
3. Open the XFp 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 μ L of sterile water.
6. Fill the moats around the outside of the wells with 400 μ L per chamber.
7. Lower the sensor cartridge onto the utility plate submerging the sensors in water.
8. Verify the water level is high enough to keep the sensors submerged.
9. Place assembled sensor cartridge and utility plate in a non-CO₂ 37°C incubator overnight.
10. 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 utility plate from 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 μ L of pre-warmed Seahorse XF Calibrant.
5. Fill the moats around the outside of the wells with 400 μ L of calibrant per chamber.
6. Lower the sensor cartridge onto the utility plate submerging the sensors in calibrant.
7. Place assembled sensor cartridge with utility plate in a non-CO₂ 37°C incubator for 45 – 60 minutes prior to loading drug ports of the sensor cartridge.