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How to Hydrate an Agilent Seahorse XFp Sensor Cartridge

For use with the Agilent Seahorse XFp Analyzer

Introduction

An important component of the Agilent Seahorse 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 O_2 concentration over time to calculate rates. For proper function, the sensors must be thoroughly hydrated before use. The following procedure describes how to hydrate and prepare the sensor cartridges for the assay. This method is designed to prevent bubble formation under the sensors during hydration, which otherwise can impact XF data quality and accuracy.

Materials

Agilent Seahorse XFp FluxPak containing:

1. Extracellular Flux Cartridges box:
    a. Sensor cartridge (12x)
    b. Utility plate (12x)
    c. Cartridge lid (12x)
2. Cell Culture Miniplates box:
    a. Agilent Seahorse XFp Miniplate with lid (12x)
3. Agilent Seahorse XF Calibrant (100 mL)

Additional items required (not included):

1. 200 µL Multichannel pipette
2. 10 mL Conical tubes
3. Cell culture grade sterile water
4. Non-CO_2 incubator at 37 °C

Procedure

Day prior to assay:

1. Aliquot at least 5 mL of XF Calibrant into a 15 mL conical tube.
2. Place this XF Calibrant in a non-CO_2 37 °C incubator overnight.
3. Remove a three-pack of cartridges from the Extracellular Flux Cartridges box. Remove the foil seal from the tub(s) that will be used.
4. Separate the utility plate and Agilent Seahorse Sensor Cartridge (Figure 1). Place the sensor cartridge upside down on the lab bench (Figure 2).
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.
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. To prevent evaporation of the water, the incubator should be 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. 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 the utility plate in a non-CO_2 37 °C incubator for 45–60 minutes prior to loading drug ports of the sensor cartridge.

Agilent Seahorse XFp Carrier Trays are included with each instrument. These carriers can hold two XFp Cartridge/Miniplate Assemblies or three miniplates without cartridges. They provide easier handling and incubation of plates and cartridges as well as compatibility with microplate-based equipment.

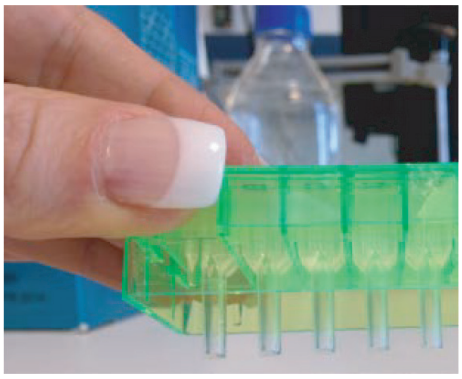

Figure 1. Sensor cartridge being lifted from utility plate.

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

Figure 3. Agilent Seahorse XFp Carrier Tray holding two cartridges supported by utility miniplates.

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