User Guide

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How to Hydrate an Agilent Seahorse XFe96 Sensor Cartridge

For use with Agilent Seahorse XFe96/XF96 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 that detects changes in both pH and O_2 concentration over time to calculate rates. In order for the sensors to function correctly, they must be thoroughly hydrated.

This document describes the method for preparing a sensor cartridge before running an XF 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 XFe96 FluxPaks containing:

1. Agilent Seahorse XFe96 Extracellular Flux Assay Kit:
   a. Cartridge Lid
   b. Sensor Cartridge
   c. Utility Plate
2. Agilent Seahorse XF96 Cell Culture Microplates
3. Agilent Seahorse XF Calibrant (500 mL)

Also required, but not included:

1. 200 µL pipettor and tips
2. 50mL conical tubes
3. Cell culture grade sterile water
4. Non-CO_2 37°C incubator

Procedure

Day prior to assay

1. Aliquot at least 20 mL of Seahorse XF Calibrant into a 50 mL conical tube.
2. Place this in a non-CO_2 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 the water
7. Verify the water level is high enough to keep the sensors submerged.
8. Place assembled sensor cartridge and utility plate in a non-CO_2 37°C incubator overnight. To prevent evaporation of the water, 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 the incubator.
2. Place the sensor cartridge 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 pre-warmed XF Calibrant.
5. Lower the sensor cartridge onto the utility plate, submerging the sensors in calibrant.
6. Place assembled sensor cartridge with utility plate in a non-CO_2 37°C incubator for 45 – 60 minutes prior to loading the injection ports of the sensor cartridge.

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