Loading the Seahorse XFp Sensor Cartridge with Compounds

Introduction

A key feature of the Seahorse XFp Analyzer is its ability to inject compounds during the assay and see results in real time. This is accomplished by dispensing compounds that have been loaded into injector ports within the cartridge prior to placement in the instrument. This procedure describes the loading process and is intended for use following overnight cartridge hydration.

Seahorse XFp Carrier Trays are included with each instrument. These carriers can hold **two** (2) XFp cartridge/miniplate assemblies or **three** (3) miniplates without cartridges. They provide easier handling and incubation of the cartridge while in a non-CO_2 incubator or while loading the cartridge on a lab bench. The procedure given below can be performed with or without the Seahorse XFp Sensor Cartridge inserted in the carrier.

Figure 1 | Seahorse XFp Carrier tray holding 2 cartridges supported by miniplates.

Best Practices for Successful Compound Loading

1. Each series of ports must contain the same volume (For example, all A ports must be filled with the same volume; all B ports must be filled with the same volume, etc.).
2. All ports of a given letter designation, including ports belonging to Background Correction or blank wells, need to have liquid loaded into them used to ensure proper injection in all wells.
3. Compounds should be diluted in prepared and pH-adjusted assay medium before being loaded into the sensor cartridge. Refer to the document “Media Preparation” for details.
   Remember: No BSA or serum in the ports!
4. The hydrated Seahorse XFp Sensor Cartridge must remain in the utility plate, and be placed flat on the work surface throughout the loading procedure. Do not lift or angle the plate/cartridge away form the work surface while loading.
5. Handle the Seahorse XFp Sensor Cartridge very carefully. Use the Seahorse XFp Carrier Tray for handling and incubating the sensor cartridge prior to placement in the instrument. Avoid carrying the cartridge long distances. To mitigate the accidental discharge of compounds prior to starting the assay, it is best to load the injection ports while in close proximity to the Seahorse XFp Analyzer.

Port Loading Procedure

Before starting the compound loading process, have the compounds prepared and a plan for which compounds go into which ports. The recommended injection volume range for the Seahorse XFp Sensor Cartridge is 20-30 µL.

Note: The hydrated XFp assay cartridge must remain in the utility plate and be placed flat on the work surface throughout the loading procedure. Do not lift or angle the plate away from the bench while loading. Hold the base of the utility plate whenever handling the cartridge to avoid triggering discharge from the injection ports.

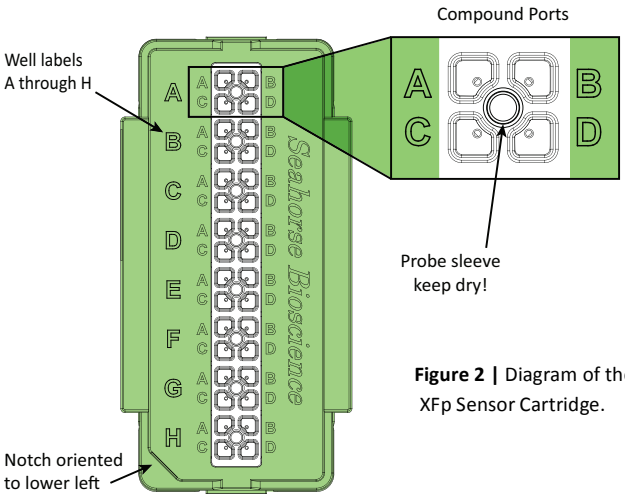

Figure 2 | Diagram of the Seahorse XFp Sensor Cartridge.

STEP 1

After preparation of compounds in the assay medium of choice, warm them to 37°C.
Note: It is Strongly recommended that injected compounds be at pH 7.35 – 7.4 at 37°C prior to loading into the injection ports.

STEP 2

Orient the Seahorse XFp Assay Cartridge: Place well labels (letters A-H) to the left. The triangular notch will be in the bottom left-hand corner. See Figure 2.

STEP 3

Using a p100 or p200 µL pipette[1], make sure the tip is securely fitted onto the pipette and aspirate the desired volume of compound to be injected.
Note: See recommendations for pipettes and tips on the next page.

[1] *Note: Automated pipettes are generally not recommended for cartridge loading, as they may lead to compound leakage through the bottom of the ports.*

STEP 4

Position the pipette tip (filled with your compounds for injection) into the desired port – see diagram in Figure 3. Orient the tip at a very slight angle (<5°) and lower the tip as far as it will go without resistance into the port. Do not force the tip into the port.

STEP 5

Dispense the compound solution into the port gently. Withdraw the tip from the port carefully. Avoid creating air bubbles. **Important!** Do NOT tap any portion of the cartridge in an attempt to alleviate air bubbles. This may cause compound leakage from the injection port.

STEP 6

Position yourself at eye level with the cartridge and visually inspect the injection ports for even loading. Make sure there are no residual drops on top of the cartridge. Record the position of any ports, which appear uneven for later data analysis. Once all compounds have been loaded according to your experimental design, carefully transfer the cartridge/utility plate assembly to the Seahorse XFp Analyzer to start calibration.

IMPORTANT: Remove the plate lid before inserting the cartridge into the Seahorse XFp Analyzer.