

# Loading the Agilent Seahorse XFp Sensor Cartridge Injection Ports

## Basic Procedure

### Introduction

A key feature of the Agilent Seahorse XFp Analyzer is its ability to inject compounds during the assay and see results in real time. This is accomplished by dispensing solutions 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.

Agilent 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<sub>2</sub> incubator or while loading the cartridge on a lab bench. The procedure given below can be performed with or without the XFp Sensor Cartridge inserted in the carrier.



Figure 1 | 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 a solution loaded into them used to ensure proper injection in all wells.
3. Compounds should be diluted in pH-adjusted assay medium before being loaded into the sensor cartridge (unless specified otherwise). For further details, consult the appropriate Agilent Seahorse XFp Kit/Reagent user manual. NOTE: Serum or BSA containing solutions should be not be loaded into the ports.
4. The hydrated 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 from the work surface while loading.
5. Handle the Agilent Seahorse XFp Sensor Cartridge carefully. Use the XFp Carrier Tray for handling and incubating the sensor cartridge prior to placement in the instrument. To mitigate the accidental discharge of compounds prior to starting the assay, it is encouraged to load the injection ports while in close proximity to the Agilent Seahorse XFp Analyzer.

## Port Loading Procedure

Recommended Injection Solution Volumes for 10X dilution upon injection, starting with a miniplate well volume of 180  $\mu$ L assay medium:

1. Port A: 20  $\mu$ l
2. Port B: 22  $\mu$ l
3. Port C: 25  $\mu$ l
4. Port D: 28  $\mu$ l

The composition, sequence and number of ports utilized will depend on the assay design.

### Step 1

After preparation of injection solutions diluted in assay medium, warm to 37°C.

NOTE: It is recommended that injection solutions be at pH 7.35 - 7.4 at 37°C prior to loading into the injection ports.



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## Step 2

Orient the Agilent 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  $\mu\text{L}$  pipette<sup>1</sup>, aspirate the desired volume of solution to be injected.

## Step 4

Position the pipette tip into the desired port. Orient the tip at a very slight angle ( $<5^\circ$ ) and lower the tip partially into the port. Do not force the tip down the bottom of the port.

## Step 5

Dispense the compound solution into the port gently. 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

Visually inspect the injection ports for even loading. Make sure there are no residual drops on top of the cartridge. Once all injection solutions have been loaded according to the experimental design, carefully transfer the cartridge/utility plate assembly to the Agilent Seahorse XFp Analyzer to start calibration.

**IMPORTANT:** Ensure the lid is off before inserting the cartridge into the XFp Analyzer.

<sup>1</sup>. Note: Automated pipettes are generally not recommended for cartridge loading, as use may lead to compound leakage through the bottom of the ports.

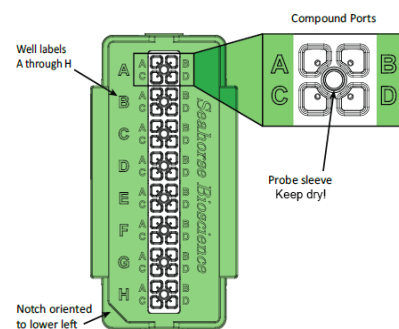


Figure 2 | Diagram of the Agilent Seahorse XFp Sensor Cartridge.

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