



Loading the Agilent Seahorse XF24 Sensor Cartridge Injection Ports

Basic Procedure

A key feature of the Agilent Seahorse XF24 Analyzer is its ability to inject reagents 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.

Recommended injection volume is 50 -100 μ L.

Recommended Injection Solution Volumes for 10X dilution upon injection, starting with a microplate well volume of 500 μ L assay medium:

1. Port A: 50 μ L
2. Port B: 62 μ L
3. Port C: 69 μ L
4. Port D: 75 μ L

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



Requirements for Proper Port 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 wells, including Background Correction or blank wells, require solution loaded in the ports being used to ensure proper injection in all wells.
3. All compounds should be diluted with the appropriate assay media before being loaded into the sensor cartridge. For further details, consult the appropriate Agilent Seahorse XF Kit/Reagent user manual.
NOTE: Serum or BSA containing solutions should not be loaded into the ports.
4. The hydrated 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 XF24 cartridge carefully. Hold the base of the utility plate when transporting a cartridge. To mitigate the accidental discharge of compounds prior to starting the assay, the best practice is to hydrate the cartridge and load the injection ports adjacent to the Agilent Seahorse XF24 Analyzer.

Loading the Sensor Cartridge with compounds:

Step 1

Pre-warm injection compounds to 37°C.

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

Step 2

Remove the cartridge lid.

Step 3

Orient the Agilent Seahorse XF24 Assay Cartridge:

When preparing for compound injections, you must identify the injection ports. The XF24 Cartridge has four injection ports, labeled A, B, C, and D as shown in the right image. Orient the Sensor Cartridge with the Row Labels on the left (A-D), and the bar code on the right. The triangular notch will be on the bottom left corner.

D	C
B	A



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Step 4

Load the XF24 Cartridge.

Using a p200 or a multichannel pipette, load the desired volume of injection solution into the appropriate injection ports using the following technique:

1. Hold the tips at a 45° angle.
Place the tips halfway into the injection ports with the bevel of the tip against the opposite wall of the injection port.
 - a. Do NOT insert the tips completely to the bottom of the injection ports as this may cause compound leakage through the port.
2. Gently and completely load the compounds into the ports.
 - a. It is important to avoid air bubbles.
 - b. However, do NOT tap any portion of the cartridge in an attempt to alleviate air bubbles. This may cause compound leakage through the injection port.
 - c. Automated pipettes are generally not recommended for cartridge loading, as they may lead to compound leakage through the bottom of the ports.



Step 5

Inspect the injection ports and calibrate cartridge. Visually inspect the injection ports for even loading. The liquid should be in the port, make sure there are no residual drops on top of the cartridge. Once all compounds have been loaded according to the experimental design, carefully transfer the cartridge (together with the utility plate) to the Agilent Seahorse XF Analyzer to start calibration prior to the assay.

Learn more
www.agilent.com/en-us/promotions/seahorse-xf-technology

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