

Loading the Agilent Seahorse XF^e96 Sensor Cartridge Injection Ports

Basic Procedure

A key feature of the Agilent Seahorse XF^e96/XF96 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 20-30 μ L.

Recommended Injection Solution Volumes for 10X dilution upon injection, starting with a microplate well volume of 180 μ L assay medium:

1. Port A: 20 μ l
2. Port B: 22 μ l
3. Port C: 25 μ l
4. Port D: 28 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, need to have solution loaded in the ports being used to ensure proper injection in all wells.
3. All compounds/reagents 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 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 XF^e96 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 XF^e96/96 Analyzer.

Loading the Sensor Cartridge with compounds:

NOTE: The hydrated 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.

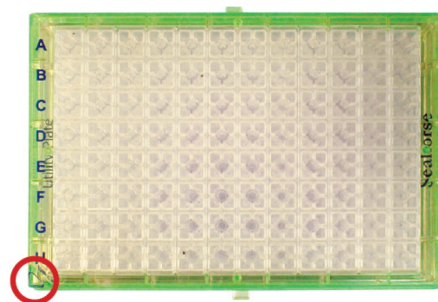
Step 1

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

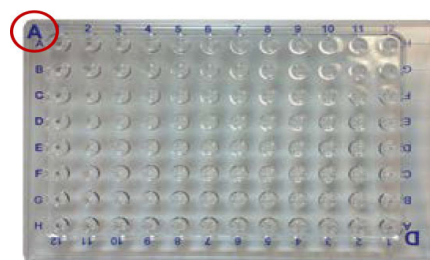
Orient the Agilent Seahorse XF Assay Cartridge. Place row labels (lettered A-H) to the left. The triangular notch (circled in red) will be in the bottom left-hand corner.



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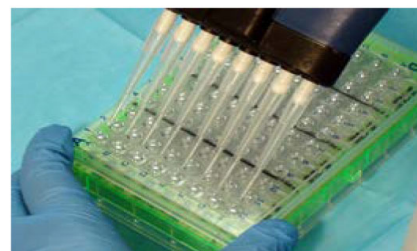
Step 3

Place the A/D loading guide flat on top of the assay cartridge. Orient the loading guide so the letter 'A' (circled in red) is located in the upper left-hand corner. Use your fingertips to hold the outside edges of the loading guide to stabilize during loading so pipette tips do not dislodge the loading guide.



Step 4

Using a 10-100 μL multichannel pipette, make sure the tips are securely fitted onto the pipette. Position the pipette tips (filled with your compounds for injection) into the desired column in the loading guide, and orient the tips at a very slight angle ($<5^\circ$). Insert the tips as far as they will go without resistance into the holes and dispense the compound. Do not force the tips completely into the holes.



NOTE: See recommendations for pipettes and tips below. Automated pipettes are not recommended for cartridge loading, as they may lead to compound leakage through the bottom of the ports.

Step 5

Dispense the compounds into the ports gently. Withdraw the tips from the ports carefully, stabilizing the loading guide throughout the procedure. Avoid creating air bubbles. 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

Switch to the B/C loading guide. Orient with the letter 'B' (circled in red) in the upper left-hand corner. Repeat loading procedure outlined in steps 3-5 for 'B', 'C' and 'D' injection ports, using the appropriate loading guides. Remove and discard loading guide(s).



Step 7

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.

IMPORTANT: Remove all loading guides and plate lids before inserting the cartridge into the Agilent Seahorse XF Analyzer.



Learn more

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

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