User Guide

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Loading the Agilent Seahorse XFe96 Sensor Cartridge Injection Ports

Basic Procedure

A key feature of the Agilent Seahorse XFe96/XF96 Analyzer is the ability to inject solutions during the assay and observe changes in respiration or glycolytic rates in real time. This is accomplished by pipetting solutions into the Injection Ports on the XF Sensor Cartridge prior to beginning an XF assay. This procedure describes the injection port loading process which is performed the day of your assay following overnight XF Sensor Cartridge hydration.

Recommended injection volume is 20-30 µL for XFe96/XF96 Analyzers.

Recommended Injection Solution Volumes for 10X dilution upon injection, starting with a microplate well volume of 180 µL assay medium (Figure 1):

1. Port A: 20 µl
2. Port B: 22 µl
3. Port C: 25 µl
4. Port D: 28 µl

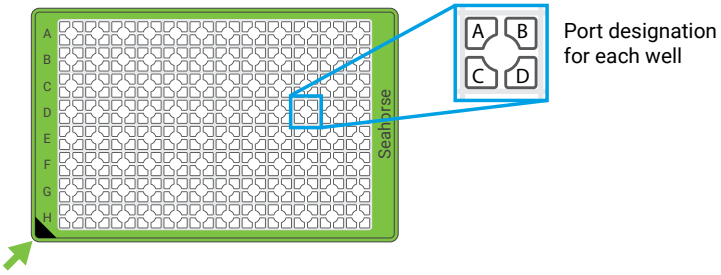

Figure 1. Recommended injection volumes and port designation for each well.

The composition and number of ports utilized will depend on your assay design.

Requirements for Injection Port Loading

1. To ensure proper injection in all wells during your assay, each series of ports must contain the same injection volume (for example, all Port A injection ports must be filled with the same injection volume across the entire XF Sensor Cartridge). This applies to Background Correction wells also.
2. All compounds/reagents should be diluted with the appropriate assay media before being loaded into the sensor cartridge, unless noted otherwise. For further details, consult the appropriate Agilent Seahorse XF Assay Kit/Reagent user manual.

   NOTE: Serum or BSA containing solutions should not be loaded into the ports.
3. The hydrated XF Sensor Cartridge must remain in the XF Utility Plate, placed flat on the work surface throughout the loading procedure. Do not lift or angle the XF Utility Plate/XF Sensor Cartridge away from the work surface while loading.
4. Handle the XFe96 sensor cartridge carefully. Hold the base of the XF Utility Plate when transporting an XF Sensor Cartridge. To mitigate the accidental leaking of injection solutions prior to starting the assay, load the injection ports in close proximity to the Seahorse XFe96/96 Analyzer.
5. A manual or automatic pipet may be used. Ensure any pipet is properly calibrated to accurately dispense volumes of 20.0 to 30.0 µL.
6. The port loading guide, provided with each XF Sensor Cartridge, can ensure the desired port is loaded. Use of the port loading guide is encouraged, but not required. The top of the XF Sensor Cartridge can be easily marked with a laboratory pen to provide a visual guide. Avoid marking any barcodes or labels on the XF Utility Plate or XF Sensor Cartridge.

Loading the XFe96 Sensor Cartridge with injection solutions

NOTE: The hydrated XF Sensor Cartridge must remain in the XF Utility Plate, flat on the work surface throughout the loading procedure. Do not lift or angle the XF Utility Plate/XF Sensor Cartridge away from the work surface while loading. Hold the base of the XF Utility Plate when handling the XF Sensor Cartridge to avoid triggering any leaking of solutions from the injection ports.

Step 1

Prepare and warm injection solutions to 37°C.

NOTE: Injected solutions should be at pH 7.3 - 7.4 at 37°C prior to loading into the injection ports. Diluting compounds in Agilent Seahorse XF DMEM, pH 7.4 or XF RPMI, pH 7.4 will facilitate accurate pH of injection solutions.

Step 2

Orient the XFe96 Sensor Cartridge as shown in Figure 2. Place row labels (lettered A-H) to the left. The triangular notch (green arrow) will be in the bottom left-hand corner (Figure 2).

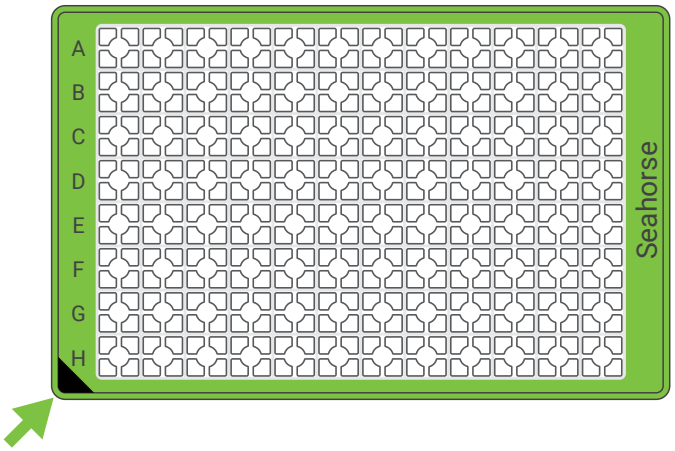

Figure 2 XFe96 Sensor Cartridge orientation.

Step 3

Place the A/D port loading guide (blue lettering) flat on top of the sensor cartridge. Orient the port loading guide so the letter 'A' (Figure 3) is in the upper left-hand corner. Use your fingertips to hold the outside edges of the port loading guide to stabilize during loading and to prevent pipette tips from dislodging the port loading guide (Figure 5).

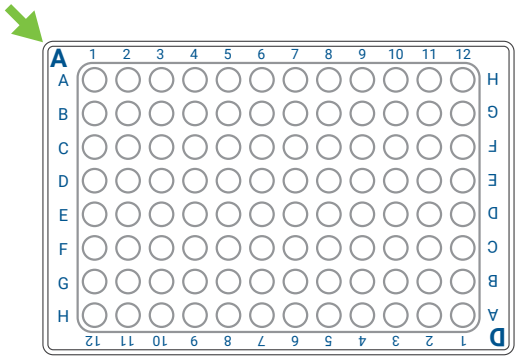

Figure 3 Orienting the A/D port loading guide.

If you are not using a port loading guide, port A is the upper-left port in each well quadrant (Figure 4).

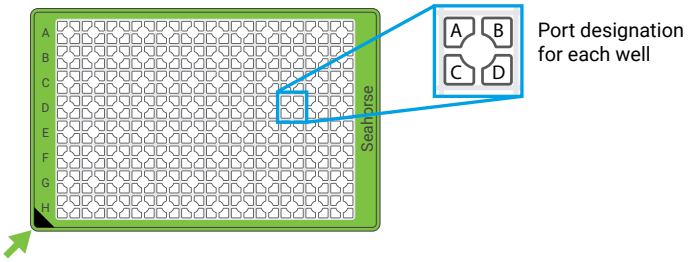

Figure 4 XFe96 Sensor Cartridge Port designations.

Step 4

Using a multi-channel pipette, first ensure tips are securely fitted onto the pipette before pipetting injection solutions. Position the pipette tips (filled with your injection solutions) into the desired column in the port loading guide, and orient the tips at a very slight angle less than 5° (Figures 5 and 6d). Insert the tips as far as they will go without resistance into the holes and dispense the injection solutions. Do not force the tips completely into the holes of the port loading guide (Figure 6c).

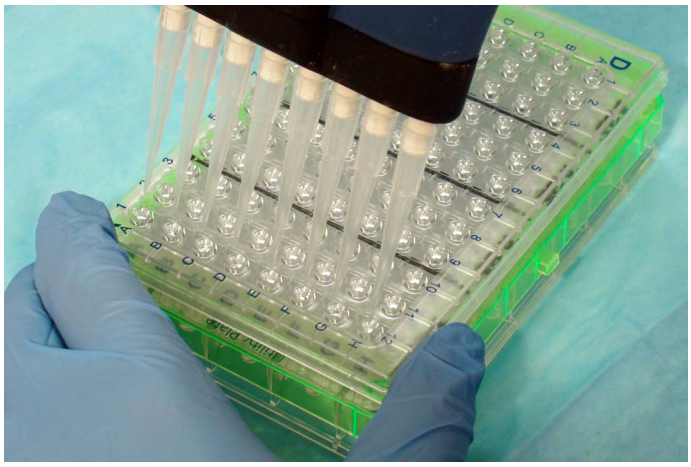

Figure 5 Dispensing Injection Solutions using the port loading guide.

When not using a port loading guide, position the pipette tips as shown in Figure 6e. Do not fully insert the pipet tips into the port as this may result in accidentally forcing injection solutions through the port orifice (Figure 6c).

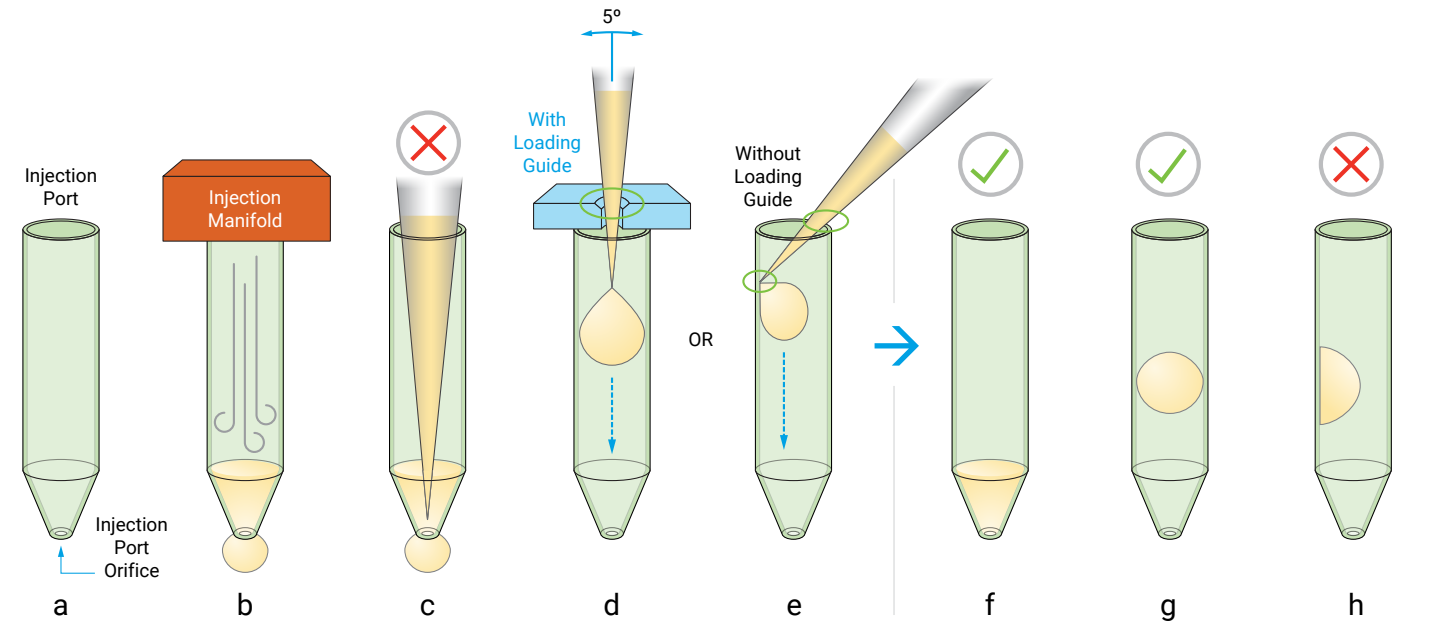

Figure 6. Injection Port Mechanism and Proper Port Loading Techniques- **(a)** Cross section of a single XF injection port. Note the open orifice at the bottom of the port. Injection solutions are retained in the ports via surface tension. **(b)** Pressurized air is used to force the solution from the port during an injection. **(c)** Do NOT insert pipettes tips fully into the ports if not using a loading guide. **(d)** If using the XF Port Loading Guide, place the pipette tips vertically into the Port Loading Guide holes as shown. **(e)** If not using the XF Port Loading Guide, then allow the pipette tips to gently touch the inside of the ports, resting the side of the pipette tips on the port rims. **(f, g)** Acceptable positions of injection solution in port after loading. **(h)** Unacceptable position of injection solution in port after loading.

Step 5

After dispensing the injection solutions into the ports, withdraw the tips from the ports carefully, holding the port loading guide throughout the procedure. Remove the A/D port loading guide and set aside for loading port D (if needed). Do not tap any portion of the XF Sensor Cartridge in an attempt to remove air bubbles from an injection port. This can cause unintended leaking of injection solutions from the injection ports.

NOTE: Forcefully dispensing injection solutions can overwhelm surface tension at the injection port opening and result in leaking of the solution from the injection port.

Step 6

Switch to the B/C port loading guide (red lettering). Orient with the letter 'B' (green arrow, Figure 7) in the upper left-hand corner, and place on top of the XF Sensor Cartridge. Repeat loading procedure outlined in steps 3-5 for 'B', and injection ports. If your experimental design requires an injection from Port D, remove the B/C port loading guide and place the A/D port loading guide on the XF Sensor Cartridge. Repeat loading procedure outlined in steps 3-5 for injection port D.. Remove and discard loading guide(s) once the entire XF sensor cartridge has been loaded.

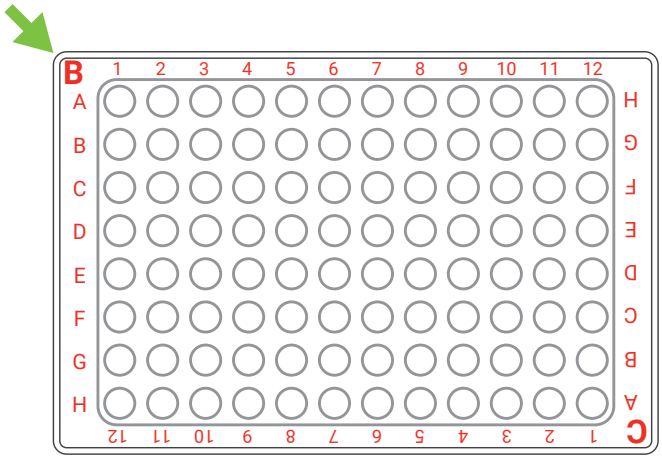

Figure 7 Orienting the B/C port loading guide.

Step 7

Visually inspect the injection ports for even loading (Figure 8). The solution should be in the port; make sure there are no residual drops on top of the XF Sensor Cartridge. Once all injection solutions have been loaded according to your experimental design, carefully transfer the XF Sensor Cartridge (together with the XF Utility Plate) to the Seahorse XFe96/XF96 Analyzer to start your assay.

IMPORTANT: Remove all port loading guides and lids for the XF Sensor Cartridge and XF Utility Plate before placement into the Analyzer.

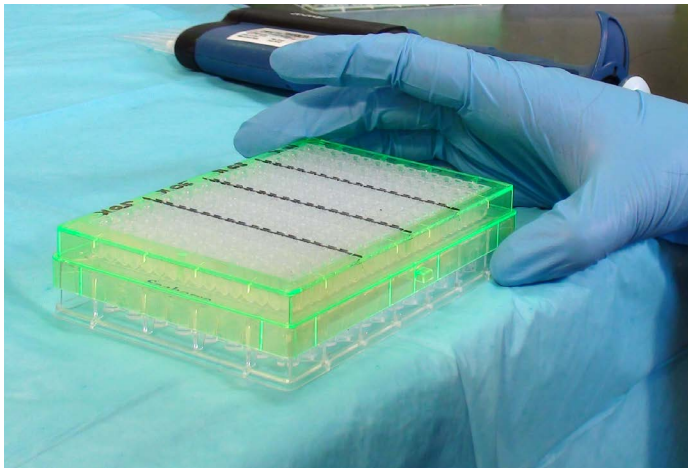

Figure 8 Visual verification of injection port loading.

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