Loading the XF^{e}96 Sensor Cartridge with Compounds

The XF^{e}96 Sensor Cartridge Loading Guide will help ensure consistent and accurate cartridge loading for assays on the XF^{e}96 Extracellular Flux Analyzers. This procedure is intended for use following cartridge hydration.

Recommended injection volume is 25 µL. *See Recommended Volumes for XF^{e}96*

Requirements for Proper 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 wells, including Background Correction or blank wells, need to have vehicle or compound loaded in the port being used to ensure proper injection in all wells.

3. All compounds should be diluted with the appropriate aqueuos vehicle (such as XF Base Medium) before being loaded into the sensor cartridge. *Refer to Compound Preparation guide.*

4. The hydrated XF 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.

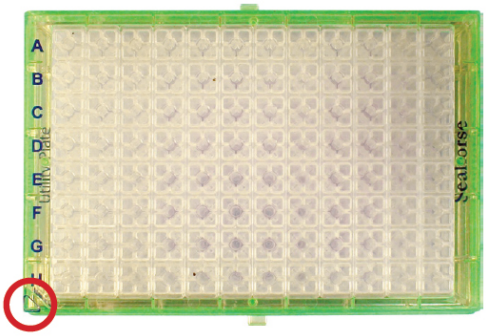
5. Handle the XF^{e}96 cartridge very carefully. Hold the base of the utility plate when transporting a cartridge. Do not hold the cartridge and utility plate between your thumb and fingers. Avoid traveling with the cartridge. To mitigate the accidental discharge of compounds prior to starting the assay, the best practice is to hydrate the cartridge and load the injection ports adjacent to the the XF^{e}96 Analyzer.

Loading the Sensor Cartridge with compounds:

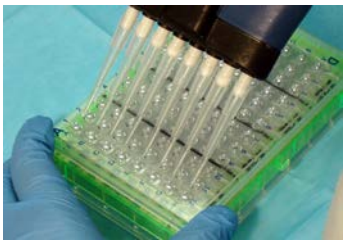
Note: The hydrated XF assay cartridge must remain in the utility plate and be placed flat on the work surface throughout the loading procedure. Do not lift or angle the plate away from the bench while loading. Hold the base of the utility plate whenever handling the cartridge to avoid triggering discharge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

STEP 1
Pre-warm injection compounds to 37^{o}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 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.

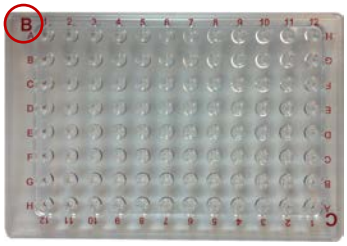


STEP 3
Place the A/D loading guide flat on top of the XF assay cartridge. Orient the loading guide so the letter 'A' (circled in red) is located in the upper left-hand corner. Use your fingertips to hold the outside edges of the loading guide to stabilize during loading so pipette tips do not dislodge the loading guide.



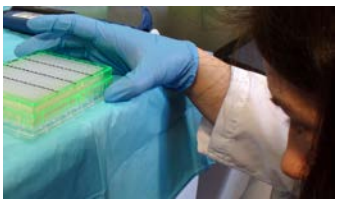
STEP 4
Using a p100 or a 10-100 µL multichannel pipette, make sure the tips are securely fitted onto the pipette. Position the pipette tips (filled with your compounds for injection) into the desired column in the loading guide, and orient the tips at a very slight angle (<5^{o}). Insert the tips as far as they will go without resistance into the holes and dispense the compound. Do not force the tips into the holes. *Note: See recommendations for pipettes and tips below. Automated pipettes are generally 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 via a single stream. 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 2-4 for 'B', 'C' and 'D' injection ports, using the appropriate loading guides.

Remove and discard loading guide(s).



STEP 7
Position yourself at eye level with the cartridge and visually inspect the injection ports for even loading. The liquid should be down at the bottom of the port, 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 (together with the utility plate) to the XF Analyzer to start calibration immediately prior to the assay.

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

Recommended Pipettes and Tips:

1. Biohit Proline Plus (10-100 µL) OR BioPette Plus (20-200 µL) with:
    a. Biohit Optifit Tip (catalog # 790351) or
    b. VWR Ultrafine Flextop Tips (Catalog # 37001-532 (USA); 7320504 (EU)) or
    c. Rainin 250 µL Tips (Catalog # RT-205)
2. Viaflo 300 µL (Dispense volume set to 25 µL, Dispense rate set to 8) with:
    a. Integra 300 µL Tips (catalog # 4433)