

Preparation of XF Glycolysis Stress Test Compounds for Running an XF^e96 Assay

Materials:

1. Reconstituted XF Glycolysis Stress Test Kit
(See basic procedure - XF Glycolysis Stress Test Kit Reconstitution)
2. XF Glycolysis Stress Test Assay Medium; pre-warmed to 37°C, pH 7.35
(See basic procedure - Preparation of Assay Media)

In the XF Analyzer, compounds are injected into the XF Cell Culture Microplate via an injection of air through drug ports located on the top of each cartridge. There are four injection channels (A, B, C, and D) and each channel distributes air evenly across all 96 wells. Consequently, when a drug port is used, **all** 96 ports of that channel must be loaded with the same volume of fluid or the injection air will distribute unevenly and result in injection failures. Use assay medium as your vehicle control. Each drug port is loaded with 25 µL of compound before the assay starts.

Seahorse recommends the following general injection and dilution scheme. This scheme takes into account the fact that each injection increases the total volume in the well and thus the concentration of each additional compound must be higher to obtain the desired final concentration. Depending on your research goals, you may wish to inject your compound of interest or various substrates before injecting the compounds of the XF Glycolysis Stress Test. In this case, you would use Port A for your compound and shift the stress test compounds to ports B, C and D.

Note that you would adjust the concentrations of the compounds as shown in the Dilution Scheme. To run the XF Glycolysis Stress Test without an additional injection, use the volumes and concentrations detailed below. Prior to running the XF Glycolysis Stress Test, the optimal oligomycin concentration must be determined empirically.

Dilution Scheme		
Assay Medium	Injection	Concentration in Port
175 µL	25 µL	8X Compound A
200 µL	25 µL	9X Compound B
225 µL	25 µL	10X Compound C
250 µL	25 µL	11X Compound D
275 µL		

Injections:

Port A: Glucose - 10 mM final concentration in the well
(8x, 80 mM stock)

Port B: Oligomycin - **Optimized** concentration in the well
(9x stock)

Port C: 2-DG – 100 mM final concentration in the well
(10x, 1 M stock)

Prepare Stock Compounds

1. Remove one aliquot each of glucose, oligomycin, and 2-Deoxy-D-glucose (2-DG) from the previously reconstituted XF Glycolysis Stress Test kit (stored at -20°C) and place on bench to thaw.
2. Pipette 3 mL of the XF Glycolysis Stress Test Assay Medium (pre-warmed to 37°C, pH 7.35) into each of two tubes (15 mL conical tubes work well).
3. For Port A, add 96 µL of 2.5 M glucose to one tube of 3 mL assay medium to make the 80 mM glucose solution for loading into the cartridge.
4. For Port B, add the appropriate volume of 5 mM oligomycin to the other tube of 3 mL assay medium (refer to table below for common optimal concentrations of oligomycin).

Oligomycin: Desired [Final] (µM)	µL of 5 mM Oligomycin aliquot	[Port] (µM) (9X)
0.5	2.7	4.5
1.0	5.4	9.0
2.0	10.8	18.0

5. For Port C, 2-DG is ready to use.
6. Load the cartridge (See Basic Procedure - Cartridge Loading).