

XF Glycolysis Stress Test Kit Preparation and Aliquoting

This basic procedure describes the process of re-suspending the compounds in the appropriate volume of DMSO or Glycolysis Stress Test assay medium to produce stocks for storage at -20°C.

Note: *The XF Glycolysis Stress Test Kit is shipped at ambient temperature. Storage at -20°C upon arrival is recommended to maximize shelf life.* Stored in this way, stock reagents have a minimum shelf life of 1 year. Prepared aliquots should be used within 3 months of reconstitution. Please refer to the XF Glycolysis Stress Test Kit Manual for further information.

NOTE: *When handling the compounds, chemical-resistant, impervious gloves should be worn and a Class II Biological Safety Cabinet should be used.*

1. Remove the XF Glycolysis Stress Test Kit from the -20°C freezer and allow the reagents to warm to room temperature (~30 minutes).
2. Preparation of Oligomycin (5 mM)
 - a. Spin down the stock vial containing powder (marked with the Seahorse logo) in a mini centrifuge for approximately 5 seconds prior to opening.
 - b. Add 180 µL of DMSO to stock vial and recap.
 - c. Vortex the vial, right side up and upside down, for 10 seconds each.
 - d. Spin down the vial in a mini centrifuge for approximately 5 seconds.
 - e. Transfer 30 µL to each provided aliquot vial. Store vials not needed immediately at -20°C.
3. Preparation of 2-Deoxy-D-glucose (2-DG) (1 M)
 - a. Re-suspend stock in 16 mL of XF Glycolysis Stress Test Assay Medium, pre-warmed to 37°C.
 - b. Adjust pH (at 37°C) to 7.35 +/- 0.05 in the stock jar or a separate beaker. (Note: Use 1 N NaOH. This will likely take only 5-20 µL.)
 - c. Adjust volume to 18 mL with XF Glycolysis Stress Test Assay Medium.
 - d. Transfer 3 mL to each provided aliquot vial.
4. Glucose is ready to use (2.5 M) and does not need to be reconstituted.
5. Record the date of re-suspension on the side of the XF Glycolysis Stress Test Kit box. Reagents that are not used immediately should be stored at -20°C. Do not subject aliquots to repeated freeze-thaw cycles.

