

Assay Tech Hints

Modifying XF^e96 Assay Parameters for use with XF^e24 and XF24 Analyzers

Introduction

Seahorse recommends the following modifications to the XF Stress Tests to be performed on XF^e24 and XF24 Analyzers. In general, assay conditions do not change other than the assay volume and cell seeding density, which will increase by **2-3x**. Use the information below as a companion to the appropriate XF Stress Test Assay Protocol and User Guide. **Please note:** *Further optimization may be required depending on parameters tested and variables modified.*

XF^e24 and XF24 Analyzers

	XF Cell Mito Stress Test	XF Glycolysis Stress Test
Injection Strategy No change from XF ^e 96 Injection Strategy	Port A: Oligomycin Port B: FCCP Port C: Rotenone + antimycin A Port D: N/A <i>*Refer to specific Assay Template Guide for reagent concentrations.</i>	Port A: Glucose Port B: Oligomycin Port C: 2-deoxyglucose (2-DG) Port D: N/A <i>*Refer to specific Assay Template Guide for reagent concentrations.</i>
Assay Media No change from XF ^e 96 Media	XF Base Medium Supplement with: 10 mM glucose 1 mM sodium pyruvate 2 mM glutamine, pH 7.4	XF Base Medium Supplement with: 2 mM glutamine, pH 7.4
Initial Assay Volume 2-3x greater than the XF ^e 96 assay volume	≥ 500 µL	
Cell Seeding Density 2-3x greater than the XF ^e 96 cell seeding density	Seed 2-3x the number of cells specified in the Assay Template Guide	
Instrument Protocol Include 2 min Wait time	Calibrate Equilibrate Basal: 3 cycles - 3 minute <i>Mix</i> , 2 minute Wait , 3 minute <i>Measure</i> Inject Port A (followed by 3 cycles) - 3 minute <i>Mix</i> , 2 minute Wait , 3 minute <i>Measure</i> Inject Port B (followed by 3 cycles) - 3 minute <i>Mix</i> , 2 minute Wait , 3 minute <i>Measure</i> Inject Port C (followed by 3 cycles) - 3 minute <i>Mix</i> , 2 minute Wait , 3 minute <i>Measure</i>	

Assay Notes

- Increase the number of cells per well 2-3x as directed by the specific Assay Template Guide.
- Verify Instrument Protocol commands are:
 - 3 minute *Mix*
 - 2 minute *Wait*
 - 3 minute *Measure*
- Repeat measurement cycles three times for Basal and after each injection.
- Analyze assay data using Seahorse Bioscience Report Generators.
 - Click here to download Report Generators:
<http://www.seahorsebio.com/support/software/stress-test-generator.php>