Modifying Assay Guides for use with Agilent Seahorse XFe24 and XF24 Analyzers

Technical Overview

Introduction

The Agilent Seahorse Assay Templates and Guides provide cell type-specific protocols for performing both Agilent Seahorse XF Stress Tests on the Agilent Seahorse XFe and XFp Analyzers. This document is intended to be used as a companion to the appropriate Seahorse XF Stress Test Assay Guide or Template. In general, assay conditions do not change other than the assay volume and cell seeding density, which will increase by 2–3x.

Note: Further optimization may be required depending on parameters tested and variables modified.

Quick Start Notes

- Increase the number of cells per well 2–3x as directed by the specific Assay Guide.
- Verify Instrument Protocol commands are:
  - 3 minute *Mix*
  - 2 minute *Wait*
  - 3 minute *Measure*
- Perform at least 3 measurement cycles for Baseline and after each injection.
- Analyze result data using the Agilent Seahorse XF Report Generators.
  - Download XF Cell Mito Stress Test Report Generator here: http://www.agilent.com/en-us/products/cell-analysis-(seahorse)/xf-cell-mito-stress-test-report-generator
  - Download XF Glycolysis Stress Test Report Generator here: http://www.agilent.com/en-us/products/cell-analysis-(seahorse)/xf-glycolysis-stress-test-report-generator

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Agilent Seahorse XFe24 and XF24 Analyzers

	XF Cell Mito Stress Test	XF Glycolysis Stress Test
Injection strategy *No change from XFe96 injection strategy*	Port A: Oligomycin Port B: FCCP Port C: Rotenone + antimycin A Port D: N/A	Port A: Glucose Port B: Oligomycin Port C: 2-deoxyglucose (2-DG) Port D: N/A
	*Refer to specific Assay Template Guide for reagent concentrations.	
Assay media *No change from XFe96 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	≥500 µL *2–3x greater than the XFe96 assay volume*	
Cell seeding density	Seed 2–3x the number of cells specified in the Assay Template Guide *2–3x greater than the XFe96 cell seeding density*	
Instrument protocol *Include 2 minute Wait time*	• Calibrate • Equilibrate • Baseline: 3 cycles 3 minute *Mix*, 2 minute *Wait*, 3 minute *Measure* • Inject Port A (followed by 3 cycles) 3 minute *Mix*, 2 minute *Wait*, 3 minute *Measure* • Inject Port B (followed by 3 cycles) 3 minute *Mix*, 2 minute *Wait*, 3 minute *Measure* • Inject Port C (followed by 3 cycles) 3 minute *Mix*, 2 minute *Wait*, 3 minute *Measure*	

For More Information

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