

## XF Cell Mito Stress Test with 3T3-L1 Cells (differentiated)

**ASSAY OVERVIEW:** General guidelines for performing the **XF Cell Mito Stress Test** assay with differentiated 3T3-L1 cells. This **Assay Guide** is for use on either XF<sup>e</sup>96, XF96, or XFp Analyzers<sup>†</sup>.

- This guide is associated with the **XF<sup>e</sup>96** Assay Template: **3T3-L1 diff-MITO-96** (.asyt file).
- This assay may be adapted for acute injections (compounds). Assign the acute injection to Port A, and reassign the injections of oligomycin, FCCP and rotenone/antimycin A to Ports B, C and D, respectively.
- Cells are to be plated at the indicated density **10-14** day(s) prior to the assay.
- The compound concentrations listed are *final* concentrations in well.
- Sample data is provided below. Absolute rates and magnitude of responses may vary based on biological and experimental variables.

**Please note:** Further optimization may be required depending on parameters tested and variables modified.

**INJECTION STRATEGY:** XF Cell Mito Stress Test  
(Final concentration in well)

- Port A: 1  $\mu$ M oligomycin
- Port B: 0.5  $\mu$ M FCCP
- Port C: 0.5  $\mu$ M rotenone + 0.5  $\mu$ M antimycin A
- Port D: N/A

**PRETREATMENTS:**

- Control Group(s)
- Experimental Group(s)

**ASSAY MEDIA:** Mito Stress Test Assay Medium

- XF Base Medium: Supplement with 10 mM glucose, 1 mM sodium pyruvate, 2 mM glutamine, pH 7.4.
- Initial Assay Volume: 180-200  $\mu$ L

**CELL SEEDING DENSITY:**

- 3T3-L1 cells differentiated into adipocyte-like cells.
- $7.0 \times 10^3$  cells/well, plated 10-14 days prior to assay.

**INSTRUMENT PROTOCOL:**

- Calibrate
- Equilibrate
- Basal: 3 cycles  
- 3 min *Mix*, 0 min *Wait*, 3 min *Measure*
- Inject Port A followed by 3 cycles  
- 3 min *Mix*, 0 min *Wait*, 3 min *Measure*
- Inject Port B followed by 3 cycles  
- 3 min *Mix*, 0 min *Wait*, 3 min *Measure*
- Inject Port C followed by 3 cycles  
- 3 min *Mix*, 0 min *Wait*, 3 min *Measure*

**XFp ANALYZER:**

- All assay parameters (assay volumes, cell seeding density and all concentrations of media components and XF Cell Mito Stress Test compounds) remain unchanged.
- Groups are limited to 2 per plate (3 wells per group).

**TYPICAL ASSAY DATA RESULTS FOR XF<sup>e</sup>96, XF96, and XFp Analyzers** (Prior to normalization)

Expected range of initial rate	Oligomycin response of initial rate*	FCCP response of initial rate*	Rotenone/antimycin A response of initial rate*
160-240 pmol O <sub>2</sub> /min	60%	270%	35%

\*The indicated values represent a percentage of the initial rate and may vary +/-20%

<sup>†</sup> For XF<sup>e</sup>24 and XF24 Analyzers, refer to **Assay Tech Hints: Modifying XF<sup>e</sup>96 Parameters for XF<sup>e</sup>24 and XF24 Analyzers**