

XF Cell Mito Stress Test with RAW 264.7 Cells

ASSAY OVERVIEW: General guidelines for performing the **XF Cell Mito Stress Test** assay with RAW 264.7 cells. This **Assay Guide** is for use on either XF^e96, XF96, or XFp Analyzers[†].

- This guide is associated with the **XF^e96 Assay Template: RAW 264.7-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 **3-5 HOURS** 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 μ M oligomycin
- Port B: 0.5 μ M FCCP
- Port C: 0.5 μ M rotenone + 0.5 μ 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 μ L

CELL SEEDING DENSITY:

- RAW 264.7 cells.
- 8.0×10^4 cells/well, plated **3-5 HOURS** 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^e96, 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*
215-325 pmol O ₂ /min	35%	140%	20%

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

[†] For XF^e24 and XF24 Analyzers, refer to **Assay Tech Hints: Modifying XF^e96 Parameters for XF^e24 and XF24 Analyzers**