

XF Cell Mito Stress Test with BT-474 Cells

ASSAY OVERVIEW: General guidelines for performing the **XF Cell Mito Stress Test** assay with BT-474 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: **BT-474-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 1 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 μM oligomycin
- Port B: 0.5 μM FCCP
- Port C: 0.5 μM rotenone + 0.5 μM 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 μL

CELLS SEEDING DENSITY:

- BT-474 cells.
- 2.0 x 10⁴ cells/well, plated 1 day 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* |
|----------------------------------|--------------------------------------|--------------------------------|--|
| 128-192 pmol O ₂ /min | 40% | 235% | 15% |

*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**