

XF Cell Mito Stress Test with Primary Rat Cortical Neurons

ASSAY OVERVIEW: General guidelines for performing the **XF Cell Mito Stress Test** assay with primary rat cortical neurons. This **Assay Guide** is for use on either XF⁹⁶, XF96, or XFp Analyzers[†].

- This guide is associated with the **XF⁹⁶** Assay Template: **Neurons-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 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 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: Neuron Mito Stress Assay Medium

- Artificial Cerebrospinal Fluid (aCSF, 120 mM NaCl, 3.5 mM KCl, 1.3 mM CaCl₂, 0.4 mM KH₂PO₄, 1 mM MgCl₂, 5 mM HEPES) + 10 mM glucose, 10 mM sodium pyruvate, pH 7.4.
- Initial Assay Volume: 180-200 μ L

CELL SEEDING DENSITY:

- Primary cortical neuron cells (rat embryo day 18)
- 1.8×10^4 cells/well, plated 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 XF⁹⁶, 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 ₂ /min	50%	175%	30%

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

[†] For XF²⁴ and XF24 Analyzers, refer to **Assay Tech Hints: Modifying XF⁹⁶ Parameters for XF²⁴ and XF24 Analyzers**.