

# Seahorse XF Cell Mito Stress Test

Mitochondrial Function Analysis for XFe96/XF Pro Analyzer

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## Assay Overview

### Purpose

The Seahorse XF Cell Mito Stress Test measures key parameters of mitochondrial function by directly measuring the oxygen consumption rate (OCR) of live cells. This assay provides comprehensive information about:

- Basal respiration
- ATP production
- Proton leak
- Maximal respiration
- Spare respiratory capacity
- Non-mitochondrial respiration

### Assay Principle

The assay uses sequential injection of mitochondrial inhibitors and uncouplers to reveal different aspects of mitochondrial function:

**Injection 1 - Oligomycin:** Inhibits ATP synthase (Complex V), revealing ATP-linked respiration

**Injection 2 - FCCP:** Uncoupler that collapses proton gradient, revealing maximal respiration

**Injection 3 - Rotenone/Antimycin A:** Complex I & III inhibitors, revealing non-mitochondrial respiration

## Timeline

1

### Day Prior (Evening)

Seed cells (10,000-20,000/well), hydrate sensor cartridge

2

### Day of Assay (Morning)

Prepare media, wash cells, prepare compounds, load ports

3

### Run Assay (~2-3 hours)

Calibration (20-30 min) + Assay run (~90-120 min)

#### Important Notes:

- Optimal cell density and FCCP concentration must be determined empirically for each cell type
- Never use vacuum aspirator when washing cells
- Do NOT seed cells in corner wells (A1, A12, H1, H12) - these are background wells
- Use compounds on the same day they are reconstituted

Dasgupta lab protocols; compiled and adapted by Roman

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