cyprotex
AN EVOTEC COMPANY

Mitochondrial Toxicity (Seahorse)

The Seahorse XF^{e}96 extracellular flux analyzer uses the mitochondrial stress test to assess mitochondrial function and cellular metabolism by detecting, in real time, the effects of a compound on oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). In the stress test, cells are exposed sequentially to oligomycin (ATP synthase inhibitor), FCCP (protonophoric uncoupler) and rotenone and antimycin A (electron transport inhibitors). This provides information on basal respiration, proton leak, maximum respiration rate and non-mitochondrial respiration. It can also be used to determine the mechanism of mitochondrial toxicity.

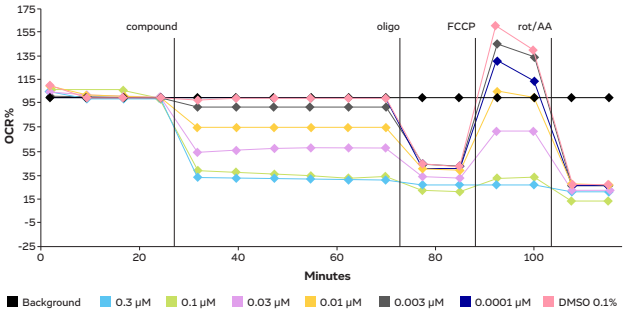
- **Flexibility:** Range of cell types available plus custom services on request
- **Sensitive Technique:** The Seahorse XF^{e}96 detects OCR and ECAR using solid state fluorescent sensors
- **Comprehensive Data:** Reporting includes AC_{50} values for OCR and ECAR, minimum effective concentrations for OCR, reserve capacity and ECAR, and a summary report
- **Knowledge and Experience:** On hand consultancy to guide you with study design, data interpretation and next steps

Proposed Screening Strategy for Mitochondrial Toxicity Assessment

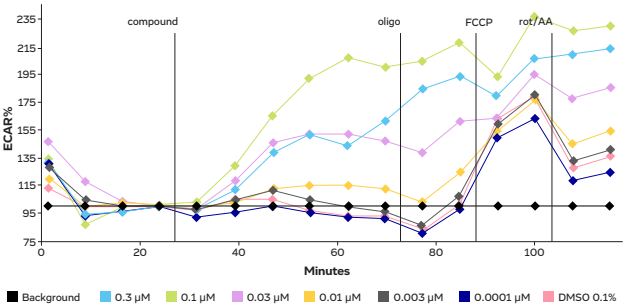
Glu/Gal Mitochondrial Toxicity or Mitochondrial Toxicity using High Content Screening	**Tier 1 Screening**
↓	
Seahorse Mitochondrial Stress Test and Mitobiogenesis	**Tier 2 Screening**
↓	
Mitochondrial Respiratory Complex Assay using Permeabilised Cells	**Mechanistic Evaluation**

Effect of Rotenone on Mitochondrial Function and Cellular Metabolism in H9c2 Cells

A). Oxygen Consumption Rate (OCR)



B). Extracellular Acidification Rate (ECAR)



The addition of rotenone, following the 4 basal readings, results in a dose-dependent decrease in OCR, and compensatory increase in ECAR. Following the addition of oligomycin, there is a decrease in OCR as expected. In the presence of FCCP, the OCR increases, and is a measure of the reserve capacity of the cells. There is a dose dependent decrease in the reserve capacity of the cells exposed to rotenone, as expected, since it is a known inhibitor of complex I of the electron transport chain.

Effect of Reference Compounds on OCR, Reserve Capacity and ECAR

Compound	Mechanism	Oxygen Consumption Rate (OCR)		Reverse Capacity		Extracellular Acidification Rate (ECAR)	
		MEC (µM)	AC_{50} (µM)	MEC (µM)	AC_{50} (µM)	MEC (µM)	AC_{50} (µM)
Rotenone	Complex I inhibitor	0.008	0.017 ↓	0.01	0.021 ↓	0.01	0.016 ↑
2-Thenoyltrifluoroacetone	Complex II inhibitor	6.5	46.4 ↓	5	17.5 ↓	48	35.8 ↑
Myxothiazol	Complex III inhibitor	0.1	0.18 ↓	3	1.8 ↓	3	1.0 ↑
Antimycin A	Complex III inhibitor	0.01	0.012 ↓	0.01	0.008 ↓	0.01	0.010 ↑
Oligomycin	Complex V inhibitor (ATP synthase inhibitor)	0.1	0.11 ↓	NR	NR	0.3	0.12 ↑
Carbonyl cyanide 3-chlorophenylhydrazone (CCCP)	Uncoupler	0.1	0.25 ↑	10	1.7 ↓	0.1	0.10 ↑
Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP)	Uncoupler	0.1	0.14 ↑	1	1.0 ↓	0.1	0.044 ↑
2,4-Dinitrophenol	Uncoupler	3	4.9 ↑	NR	NR	3	1.4 ↑
Etomoxir	ß-oxidation inhibitor	7	94.9 ↓	NR	67.9 ↓	7	NR
UK-5099	Pyruvate transport inhibitor	19.3	92.1 ↓	0.1	2.3 ↓	0.09	NR
2-Deoxyglucose	Glycolysis inhibitor	NR	NR	NR	NR	NR	NR
Methapyrilene	No evidence	NR	NR	NR	NR	NR	NR
Physostigmine	No evidence	NR	NR	NR	NR	NR	4.5 ↑
Betaine	No evidence	NR	NR	NR	NR	NR	NR
Streptomycin	No evidence	NR	NR	NR	NR	NR	NR

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