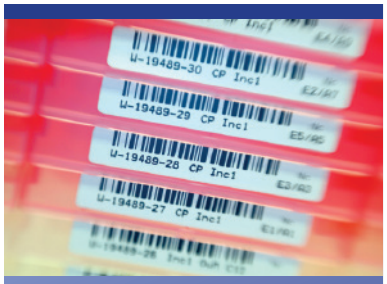
cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

Functional Mitochondrial Toxicity Assay (using Seahorse XF^{e}96 flux analyser)

Background Information



'Drug-induced mitochondrial toxicity is rapidly gaining recognition within the pharmaceutical industry as a contributor to compound attrition and post-market drug withdrawals.'

[3]Nadanaciva S and Will Y (2011) *Current Pharmaceutical Design* **17**; 2100-2112

- Impairment of mitochondrial function is increasingly implicated in the etiology of drug-induced toxicity.[1]
- The Seahorse XF^{e}96 extracellular flux analyser is used to detect, in real time, effects of compounds on oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in order to assess mitochondrial function and cellular metabolism.
- The assay uses the mitochondrial stress test to gain an insight into cellular bioenergetics and the mechanism of mitochondrial toxicity.[2]
- 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.
- As well as mitochondrial toxicity, the Seahorse XF^{e} flux analyser can be used for other applications where a shift between mitochondrial respiration and glycolysis is observed under certain pathological states (e.g., obesity, diabetes, cancer, cardiovascular disease and neurodegenerative function).

Protocol

Media Assessed
Unbuffered DMEM containing 10 mM glucose, 1 mM pyruvate and 2 mM glutamine

Cell Types Available
H9c2, Huh7, HepG2 (other custom cell lines available on request)

Test Article Concentration
7 point dose response

Quality Controls
Vehicle control
Rotenone (positive control)

Test Article Requirements
50 µL of 50 mM DMSO solution or equivalent amount of solid compound

Analysis Method
Use of solid state fluorescent sensors to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Measured using the XF^{e}96 flux analyser (Seahorse Biosciences Inc)

Data Delivery
Summary report
AC_{50} for OCR, reserve capacity and ECAR
Minimum effective concentration (MEC) for OCR, reserve capacity and ECAR

Related Services

HCS based mitochondrial toxicity assessment
Glucose/galactose mitochondrial toxicity assessment

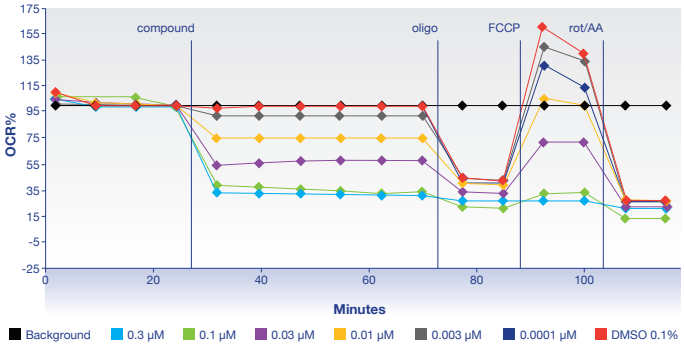
Known mitochondrial toxicants and non-toxicants were screened in the Seahorse assay.

Figure 1

Effect of rotenone on A) oxygen consumption rate and B) extracellular acidification on H9c2 cells.

The addition of rotenone following the 4 basal reading results in a dose dependent decrease in (A) OCR, and compensatory increase in (B) ECAR. Following the addition of oligomycin, there is a decrease in OCR as expected, demonstrating no increase in proton leak. In the presence of FCCP, the OCR increases, and is a measure of the reserve capacity of the cells. There is a dose dependendent 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.

A.



B.

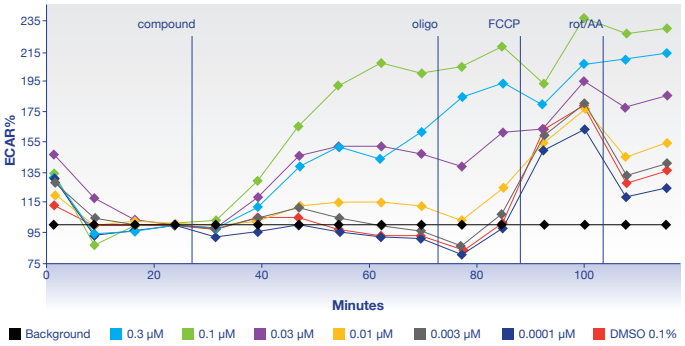


Table 1

Effect of test compounds on OCR, Reserve Capacity and ECAR

		Oxygen Consumption Rate (OCR)		Reserve Capacity		Extracellular Acidification Rate (ECAR)	
Compound	**Mechanism**	**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

NR = no response

References

[1] Dykens JA and Will Y (2007) The significance of mitochondrial toxicity testing in drug development. *Drug Discovery Today* **12**; 777-785

[2] Brand MD and Nicholls DG (2011) Assessing mitochondrial dysfunction in cells. *Biochem J* **435**; 297–312

[3] Nadanaciva S and Will Y (2011) New insights in drug-induced mitochondrial toxicity. *Current Pharmaceutical Design* **17**; 2100-2112