

## In vitro Toxicology

# Mitochondrial Respiratory Complex Assay using Permeabilised Cells

## 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.'

<sup>1</sup>Nadanaciva S & Will Y (2011)  
*Current Pharmaceutical Design*  
17; 2100-2112

- Impairment of mitochondrial function is implicated in the etiology of drug-induced toxicity.
- The Seahorse XF<sup>®</sup>96 extracellular flux analyser is used to detect, in real time, effects of compounds on oxygen consumption rate (OCR) in order to assess mitochondrial function.
- Permeabilisation of cells which leaves the mitochondrial membrane intact allows the study of mitochondrial function without the need to isolate mitochondria.
- The use of complex-specific substrates and inhibitors allows the identification of the individual complexes (complex I, complex II, complex III and complex IV) of the electron transport chain (ETC) involved in mitochondrial toxicity.
- The mitochondrial respiratory complex assay can be used in conjunction with other mitochondrial assays (e.g., the Seahorse functional mitochondrial toxicity assay, glu/gal assay or the HCS-based mitochondrial assay) to determine the potential for mitochondrial toxicity along with an understanding of the mechanism.

### Related Services

Glucose/galactose mitochondrial toxicity assay

HCS-based mitochondrial toxicity assay

Functional mitochondrial toxicity assay (Seahorse XF<sup>®</sup>96)

### Protocol

#### Cell Type

HepG2 (others available on request)

#### Analysis Platform

Seahorse XF<sup>®</sup>96 flux analyser  
(Agilent Technologies)

#### Analysis Method

Use of solid state fluorescent sensors to measure oxygen consumption rate (OCR)

#### Mechanism\*

Pyruvate respiration  
Succinate respiration  
Ascorbate respiration

#### Test Article Requirements

50  $\mu$ L of a DMSO stock solution to achieve 100x  $C_{max}$  (200x top concentration to maintain 0.5% DMSO) or equivalent amount in solid compound

#### Test Article Concentration\*

7 point dose response curve with top concentration based on 100x  $C_{max}$  or solubility limit

#### Number of Replicates\*

3 replicates per concentration

#### Quality Controls\*

Negative control: 0.5% DMSO (vehicle)  
Positive control: Assay appropriate control

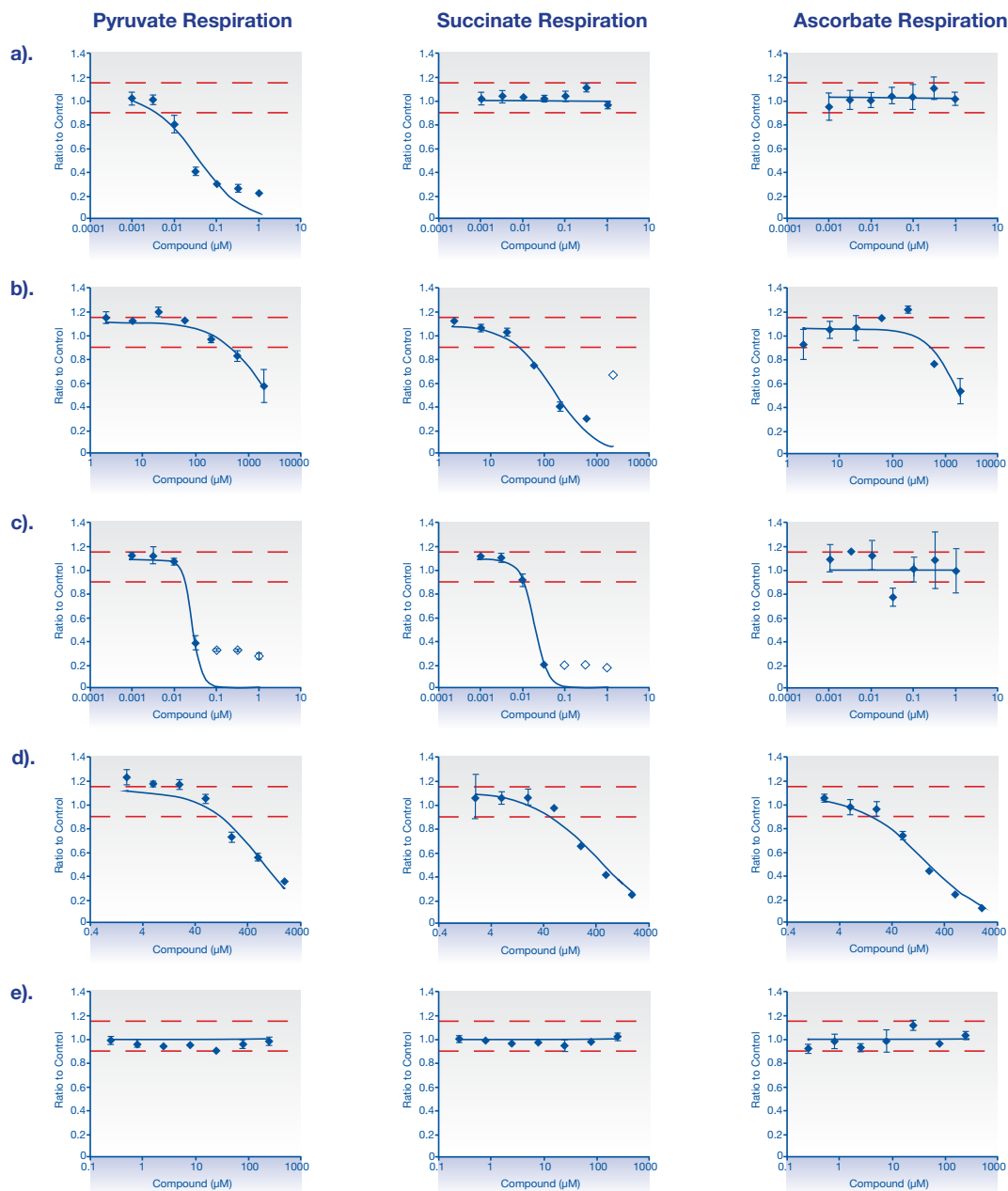
#### Data Delivery

Minimum effective concentration (MEC) and  $AC_{50}$  values with dose response curves for each measured parameter

\* Other options available on request

**Figure 1**

Representative data assessing the effects on Complex I, Complex II/III and Complex IV mitochondrial respiration on permeabilised HepG2 cells. Compounds tested were a). rotenone, b). thenoyltrifluoroacetone (TTFA), c). antimycin A, d). sodium azide and e). betaine



Known mitochondrial toxicants and non-toxicants were screened in the mitochondrial respiratory complex assay. The identified mechanisms of action were compared to those published in the literature.

The oxygen consumption rate (OCR) of permeabilised HepG2 cells was measured in the presence of an appropriate complex I substrate (pyruvate). The test compound was injected directly onto the cells and OCR determined. Following this, a complex II/III substrate (succinate) and a complex I inhibitor were injected and further measurements taken. Finally a complex IV substrate (ascorbate) and complex III inhibitor were added and a final OCR was determined.

A reduction in OCR following the addition of test compound indicates inhibition of one of the complexes of the electron transport chain. If this inhibition is overcome by the addition of an alternative substrate, it indicates the potential site of inhibition.

**Table 1**

Summary of validation data

Compound	Mechanism	Pyruvate Respiration		Succinate Respiration		Ascorbate Respiration	
		MEC (µM)	AC <sub>50</sub> (µM)	MEC (µM)	AC <sub>50</sub> (µM)	MEC (µM)	AC <sub>50</sub> (µM)
Rotenone	Complex I inhibitor	0.006	0.033	No response	No response	No response	No response
Ketoconazole	Complex I inhibitor	13.3	42.1	No response	No response	No response	No response
Carboxine	Complex II inhibitor	No response	No response	2.43	23.3	No response	No response
Thenoyltrifluoro-acetone (TTFA)	Complex II inhibitor	605	>2000	45.6	151	688	1810
Antimycin A	Complex III inhibitor	0.019	0.027	0.012	0.019	No response	No response
Sodium azide	Complex IV inhibitor	177	654	79.1	411	23.5	156
Streptomycin	No effect	No response	No response	Not response	No response	No response	No response
Betaine	No effect	No response	No response	No response	No response	No response	No response

MEC- minimal effective concentration

#### References

<sup>1</sup>Nadanaciva S and Will Y (2011) New insights in drug-induced mitochondrial toxicity. *Current Pharmaceutical Design* 17; 2100-2112