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*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.'

[1]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$^e$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$^e$96)

### Protocol

**Cell Type**
HepG2 (others available on request)

**Analysis Platform**
Seahorse XF$^e$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 µL 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

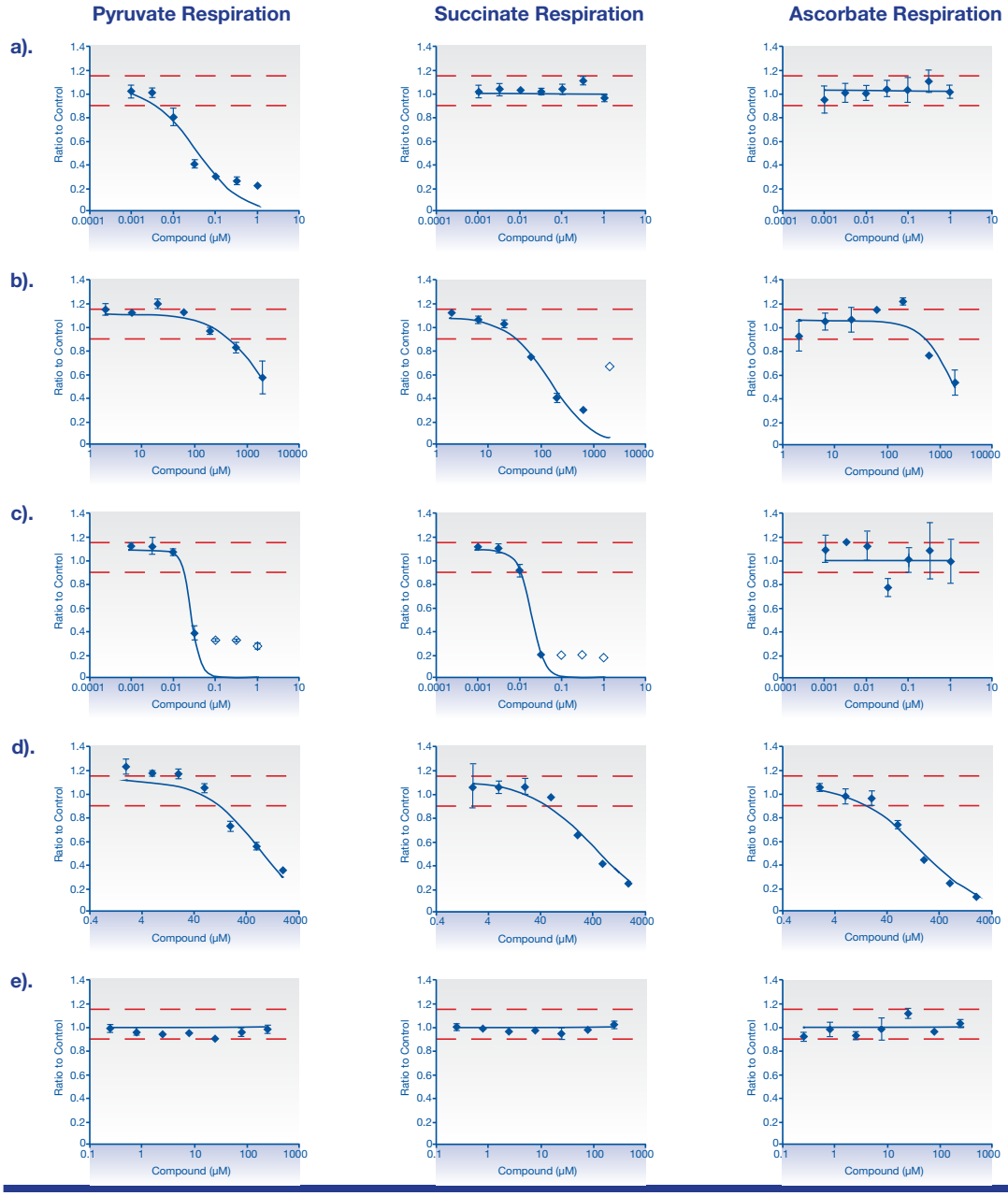
**Data Delivery**
Minimum effective concentration (MEC) and $AC_{50}$ values with dose response curves for each measured parameter

* Other options available on request

To find out more contact **enquiries@cyprotex.com**

## 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) | $AC_{50}$ (µM) | MEC (µM) | $AC_{50}$ (µM) | MEC (µM) | $AC_{50}$ (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**

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