Mitochondrial Biogenesis

Background Information

- Mitochondrial proteins are encoded by both mitochondrial and nuclear genomes. Mitochondrial biogenesis is defined as the growth and division of pre-existing mitochondria.\(^1\) Certain drugs are able to affect mitochondrial biogenesis through inhibition of mtDNA replication or mitochondrial encoded protein synthesis.

- There is certain similarity between mitochondrial biogenesis and bacterial and viral replication. As a consequence, many antibacterial and antiviral agents cause mitochondrial toxicity through effects on human mitochondrial biogenesis. In fact, the FDA suggest that all antiviral drugs should be tested for effects on mitochondrial function.\(^2\)

- Cyprotex use high content screening and fluorescently labelled antibodies to evaluate ratios of an mtDNA-encoded protein (COX-1, a subunit of Complex IV) and an nDNA-encoded protein (SDH-A, a subunit of Complex II).

- The mitochondrial biogenesis assay can be used to identify drugs cause toxicity through inhibition of mitochondrial biogenesis.

\(^1\)Moreira AC et al., (2011) In: Biosensors for Health, Environment and Biosecurity, Serra PA (Ed.) 411-444

\(^2\)Data from Moreira et al. (2011) In: Biosensors for Health, Environment and Biosecurity, Serra PA (Ed.) 411-444

Protocol

- **Method**
  - High content screening using fluorescently labelled antibodies for COX-1 and SDH-A

- **Cell Types**
  - HepG2 or HepaRG (others available on request)

- **Exposure Time**
  - 5-8 days depending on cell line and customer needs

- **Number of Concentrations**
  - 10 concentrations (n=3 wells per concentration)

- **Positive Control**
  - Chloramphenicol

- **Negative Control**
  - Lincomycin

- **Data Delivery**
  - COX-1 protein expression
  - SDH-A protein expression
  - COX-1/SDH-A protein expression ratio
  - Cell loss
Certain drugs are able to affect mitochondrial biogenesis through inhibition of mtDNA replication or mitochondrial encoded protein synthesis.

**References**

Cyprotex’s mitochondrial biogenesis assay uses high content imaging to detect COX-1 (mtDNA-encoded) and SDH-A (nDNA-encoded) protein expression. Using the ratio of COX-1/SDH-A protein expression, specific effects on mitochondrial biogenesis can be identified. The positive control for the assay, chloramphenicol (antibiotic), inhibits mitochondrial biogenesis in a dose-dependent manner (figure 1).

**Figure 1**
Dose curve for chloramphenicol in the mitochondrial biogenesis assay

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**Figure 2**
Effect of the antiviral, R1479 (4'-azidocytidine) on mitochondrial biogenesis as illustrated by the impact on COX-1/SDH-A protein expression