

Mitochondrial Biogenesis

Background Information



‘Mitochondria are the only organelles outside of the nucleus that contain their own genome and replicate itself in an independent manner from the nuclear genome.’

³Moreira AC *et al.*, (2011) In: *Biosensors for Health, Environment and Biosecurity*, Serra PA (Ed.) 411-444

- 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.

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

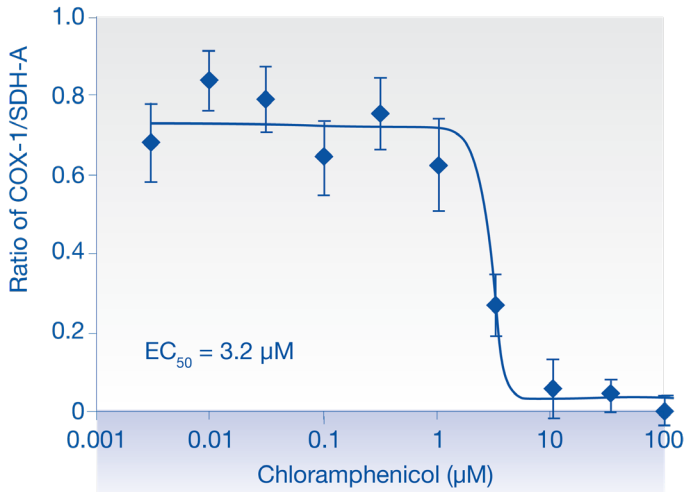
Data Delivery

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.

Figure 1

Dose curve for chloramphenicol in the mitochondrial biogenesis assay

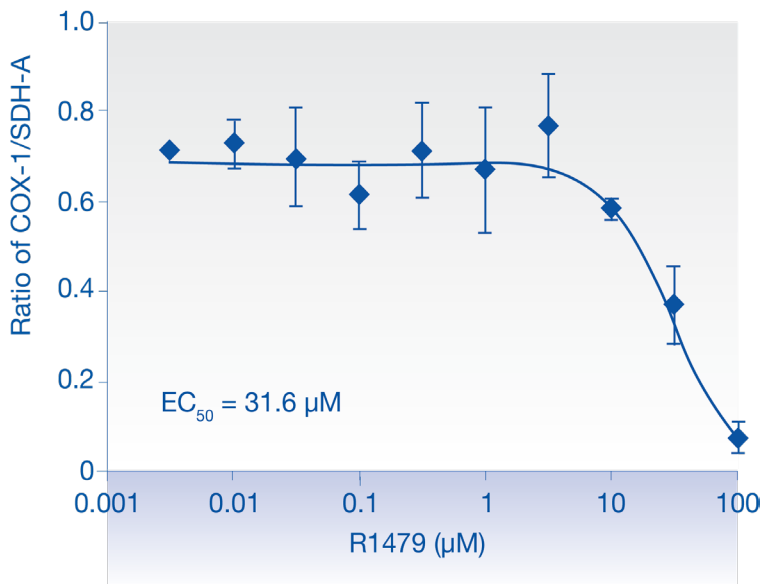


Cyprotex's mitochondrial biogenesis assay uses high content imaging to detect COX-1 (mtDNA-encoded) and SDH-A (nDNA-encoded) protein expression. Using the ratio of COX-1/SDH-A protein expression, specific effects on mitochondrial biogenesis can be identified. The positive control for the assay, chloramphenicol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

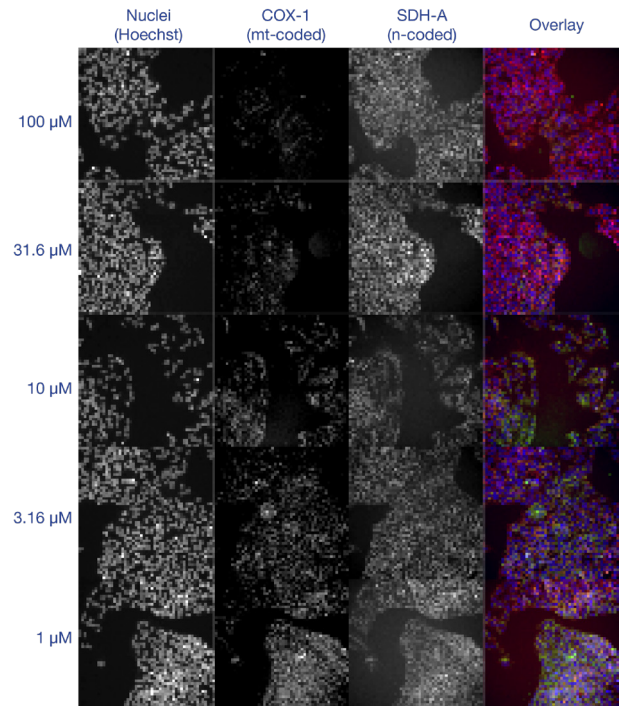
Figure 2

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

A. Dose curve for R1479



B. High content imaging for R1479



References

- Jornayvaz FR and Shulman GI (2010) Regulation of mitochondrial biogenesis. *Essays Biochem* 47; 69-84
- Food and Drug Administration. Guidance for Industry: Antiviral product development - Conducting and submitting virology studies to the agency (<https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070953.pdf>)
- Moreira AC et al., (2011) Mitochondria as a biosensor for drug-induced toxicity - Is it really relevant? *Biosensors for Health, Environment and Biosecurity*, Serra PA (Ed.); 411-444