

Human Cyt C Reductase ELISA Kit (Complex III) Profiling ELISA Kit ab124537

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Overview

Product name Human Cyt C Reductase ELISA Kit (Complex III) Profiling ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Overall	8			4.7%

Inter-assay

Sample	n	Mean	SD	CV%
Overall	6			19.7%

Sample type Tissue Extracts, Cell Lysate

Assay type Sandwich (qualitative)

Sensitivity 4 µg/ml

Range 6 µg/ml - 200 µg/ml

Assay duration Multiple steps standard assay

Species reactivity **Reacts with:** Human

Does not react with: Mouse, Rat

Product overview

Ubiquinol-cytochrome-c reductase (Complex III) is the third enzyme of the oxidative phosphorylation (OXPHOS) system within the mitochondrial inner membrane. Ubiquinol-cytochrome-c reductase is a large protein complex of approximately 250,000 MW made up of 11 different subunits. The enzyme forms a dimer in the mitochondrial inner membrane. The catalytic subunit cytochrome b is encoded on mitochondrial DNA (mtDNA). All other subunits are encoded by nuclear genomic DNA, made in the cytosol, and translocated into the organelle for assembly at the inner membrane. Complex III catalyses the pumping of protons across the inner membrane and transfer of electrons from ubiquinol to cytochrome c by a mechanism known as the Q cycle in which ubiquinol (Q) the electron carrier exists as a reduced, partially reduced or oxidized form.

ab124537 Cyt C Reductase (Complex III) human profiling kit is an in vitro enzyme-linked immunosorbent assay (ELISA) for the comparison of Complex III levels or profile in cell and tissue

lysates. The assay employs a human Complex III specific antibody coated onto microplate well plate strips.

Samples are pipetted into the wells and Complex III present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an anti-Complex III detector antibody is added. After washing away unbound detector antibody, an HRP-conjugated secondary antibody specific for the detector antibody is pipetted into the wells. The wells are again washed, an HRP substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Complex III bound. The developing blue color is measured at 600 nm.

Optionally the reaction can be stopped by adding hydrochloric acid which changes the color from blue to yellow and the intensity can be measured at 450 nm.

Species– Human. Rat and Mouse samples are not appropriate, other species are untested.

Notes Store all components at 4°C. This kit is stable for 6 months from receipt. Unused microplate strips should be returned to the pouch containing the desiccant and resealed.

Platform Microplate

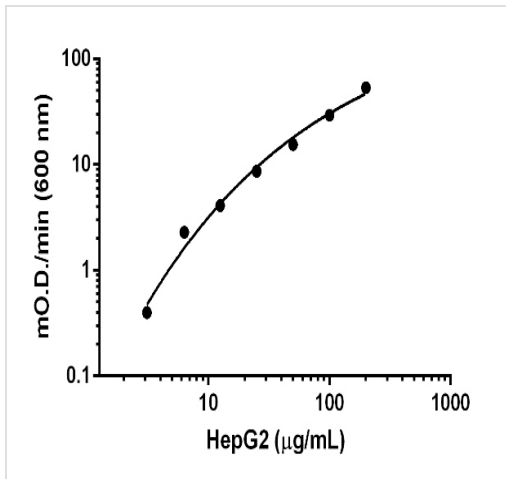
Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Blocking Buffer	1 x 6ml
10X Complex III Detector Antibody	1 x 1ml
10X HRP Label	1 x 1ml
20X Buffer	1 x 20ml
Complex III Microplate	1 unit
Extraction Buffer (ab260490)	1 x 15ml
HRP Development Solution	1 x 12ml

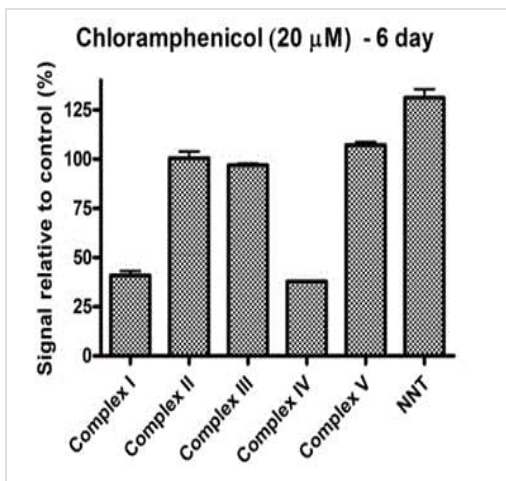
Relevance Complex II (succinate-ubiquinone oxidoreductase), one of the mitochondrial respiratory chain complexes, transfers electrons from succinate generated during the citric acid cycle to Complex III (ubiquinolcytochrome c oxidoreductase), via a mobile electron shuttle, ubiquinone. Complex III transfers electrons to Complex IV (cytochrome c oxidase) via another mobile electron shuttle, cytochrome c.

Images



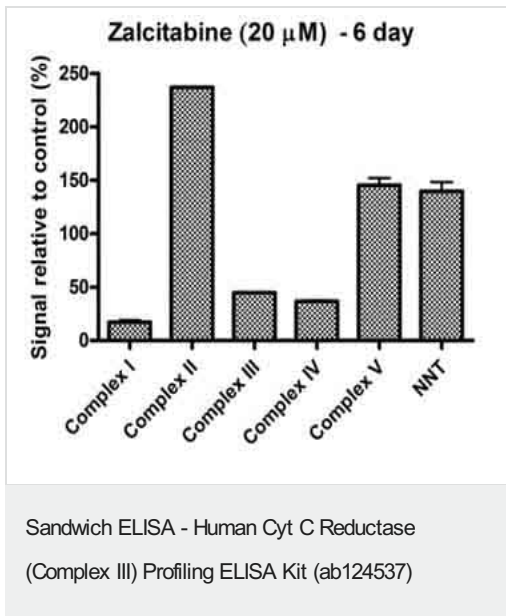
Example control sample curve of serially titrated HepG2 extract in the working range of the assay.

Sandwich ELISA - Human Cyt C Reductase
(Complex III) Profiling ELISA Kit (ab124537)



Human HepG2 cells were cultured in chloramphenicol for 6 days to ensure a significant effect on mitochondrial DNA replication and mitochondrial protein translation, respectively. The antibiotic inhibited mitochondrial protein translation and assembly of Complexes I and IV but had no significant effect on Complex II, III or V.

Sandwich ELISA - Human Cyt C Reductase
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Human HepG2 cells were cultured in NARTI Zalcitabine (ddC) for 6 days to ensure a significant effect on mitochondrial DNA replication and mitochondrial protein translation, respectively. The drug reduced mitochondrial DNA levels and hence mitochondrial protein expression. As a consequence the assembly of Complexes I, III and IV were severely affected. Note that loss of the two small mitochondrial DNA encoded subunits of Complex V (ATP synthase) does not affect overall assembly. Interestingly an increase in Complex II was induced as a consequence of I, III, IV loss possibly to up regulate mitochondrial citric acid cycle function.

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