abcam

Product datasheet

Human ATP Synthase ELISA Kit (Complex V) Profiling ELISA Kit ab124539

5 References 3 Images

Overview

Product name Human ATP Synthase ELISA Kit (Complex V) Profiling ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Overall	4			2.3%

Inter-assay

Sample	n	Mean	SD	CV%
Overall	3			3.5%

Sample type Tissue Extracts, Cell Lysate

Assay type Sandwich (qualitative)

Sensitivity 12 µg/ml

Range 12 µg/ml - 1000 µg/ml

Assay duration Multiple steps standard assay

Species reactivity
Reacts with: Human
Does not react with: Mouse, Rat

Product overview ATP synthase (Complex V) is the fifth enzyme of the oxidative phosphorylation (OXPHOS) system within the mitochondrial inner membrane. ATP synthase is a large protein complex of approximately 550,000 MW made up of 17 different subunits arranged in a membrane embedded proton translocating domain (F0 domain) and a soluble ATP synthesizing catalytic domain (F1 domain). Two membrane embedded subunits ATP6 (subunit a) and ATP8 (subunit 8) are 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. A proton gradient generated by the other enzymes of the OXPHOS system provides the energy for ATP synthesis by a rotational catalytic method known as the binding change mechanism. ATP synthase is regulated by CabI and IF1 proteins in response to calcium concentration and mitochondrial membrane potential respectively.

ab124539 ATP synthase (Complex V) human profiling kit is an in vitro enzyme-linked immunosorbent assay (ELISA) for the comparison of ATP synthase levels or profile in cell and tissue lysates. The assay employs a capture antibody specific for human ATP synthase coated onto microplate well strips.

Samples are pipetted into the wells and ATP synthase present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an anti-ATP synthase 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 ATP synthase 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.

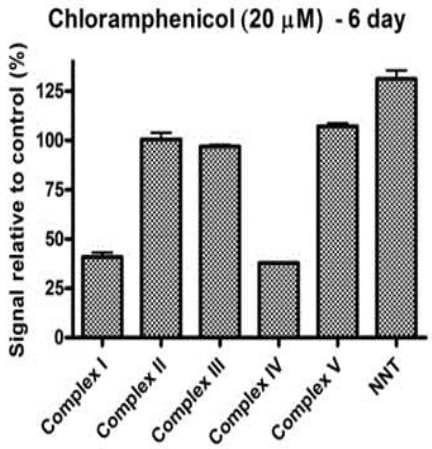
Platform Microplate

Properties

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

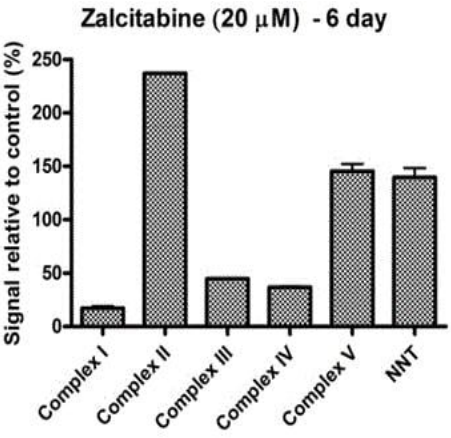
Components	1 x 96 tests
10X ATP Synthase Detector Antibody	1 x 1ml
10X Blocking Buffer	1 x 6ml
10X HRP Label	1 x 1ml
20X Buffer	1 x 20ml
ATP Synthase Microplate	1 unit
Extraction Buffer (ab260490)	1 x 15ml
HRP Development Solution	1 x 12ml

Images



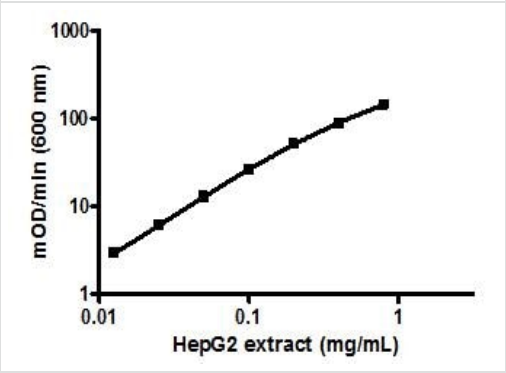
Sandwich ELISA - Human ATP Synthase (Complex V) Human Profiling ELISA Kit (ab124539)

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 ATP Synthase (Complex V) Human Profiling ELISA Kit (ab124539)

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.



Sandwich ELISA - Human ATP Synthase (Complex V) Human Profiling ELISA Kit (ab124539)

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

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