

Complex I Enzyme Activity Microplate Assay Kit (Colorimetric) ab109721

★★★★★ [4 Abreviews](#) [234 References](#) [6 Images](#)

Overview

Product name Complex I Enzyme Activity Microplate Assay Kit (Colorimetric)

Detection method Colorimetric

Sample type Cell culture extracts, Tissue

Assay type Enzyme activity

Assay time 3h 30m

Species reactivity **Reacts with:** Mouse, Rat, Cow, Human

Product overview Complex I Enzyme Activity Assay Kit (ab109721) is a kit designed for the analysis of mitochondrial OXPHOS Complex I enzyme activity from human, rat, mouse and bovine cell and tissue extracts.

Capture antibodies specific for Complex I are pre-coated in the microplate wells. Samples are added to the microplate wells which have been precoated with a specific capture antibody. After the target has been immobilized in the well, Complex I activity is determined by following the oxidation of NADH to NAD⁺ and the simultaneous reduction of a dye which leads to increased absorbance at OD=450 nm. By analyzing the enzyme's activity in an isolated context, outside of the cell and free from any other variables, an accurate measurement of the enzyme's functional state can be understood.

Complex I activity is controlled by enzyme amount and by post-translational phosphorylation at key specific regulatory residues. Cellular metabolism governs these two factors. Ultimately, the cell type and growth conditions will affect Complex I activity measurements.

Note: This activity assay measures the diaphorase-type activity of Complex I. This activity is not dependent on the presence of ubiquinone and therefore inhibitors, such as rotenone, which bind at or near the ubiquinone binding site do not inhibit this assay. However, both the activity assay and the quantity assay described here are affected by enzyme assembly deficiencies.

Review our [Metabolism Assay Guide](#) to learn about assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also about how to assay metabolic function in live cells using your plate reader.

Chinese protocol available. See protocols section below.

Notes

ab109721 is shipped at 4°C. 20X NADH and 100X Dye are shipped lyophilized. Rehydrate 20X NADH by adding 1.1 mL H₂O. Rehydrate 100X Dye by adding 0.25 mL H₂O, then vortex each thoroughly until dissolved. After hydration unused amounts of these two materials should be stored at -80°C. When planning multiple experiments, aliquot these reagents to prevent freeze thaw cycles. Store all other components at 4°C

Related products

Review the [mitochondrial assay guide](#), or the full [metabolism assay guide](#) to learn about more assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also how to assay metabolic function in live cells using your plate reader.

Platform

Microplate reader

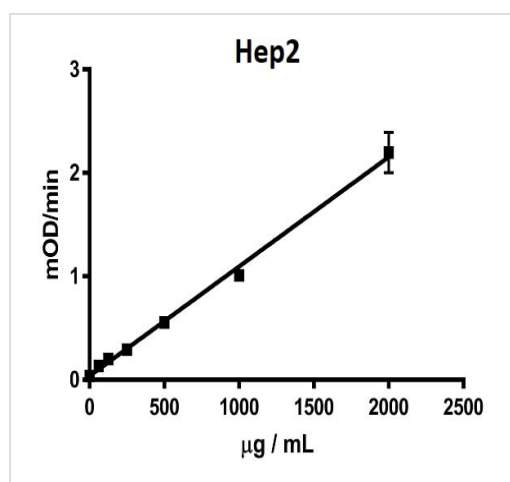
Properties

Storage instructions

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

Components	96 tests
100X Dye (lyophilized)	1 unit
10X Blocking Solution	1 x 10ml
Detergent	1 x 1ml
20X Wash Buffer	1 x 25ml
20X NADH (lyophilized)	1 unit
96-well microplate (12 strips)	1 unit

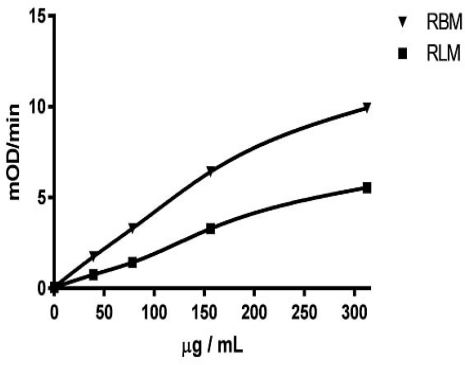
Images



ab109721 measures Complex I activity in human cultured cells within the recommended ranges given in the protocol. Example of Complex I activity measured in Hep2 cells is shown. (Note that these ranges depend on mitochondria preparation quality).

Functional Studies - Complex I Enzyme Activity
Microplate Assay Kit (ab109721)

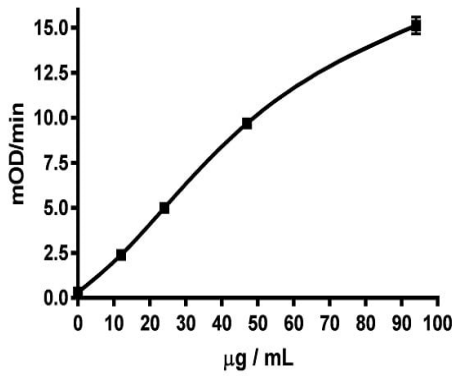
Rat Brain/Liver Mitochondria



Functional Studies - Complex I Enzyme Activity
Microplate Assay Kit (ab109721)

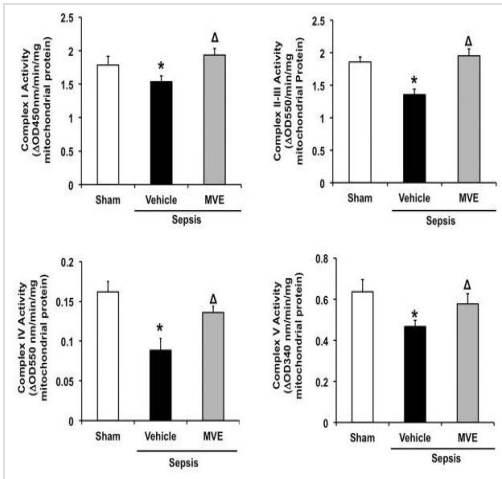
ab109721 measures Complex I activity in human and rat cultured cells but also in tissues/tissue mitochondria samples within the recommended ranges given in the protocol. Examples of Complex I activity measured in different rat tissue mitochondrial samples are shown. (Note that these ranges depend on mitochondria preparation quality).

Rat Heart Mitochondria



Functional Studies - Complex I Enzyme Activity
Microplate Assay Kit (ab109721)

Examples of Complex I activity measured in different rat tissue mitochondrial samples are shown. (Note that these ranges depend on mitochondria preparation quality).

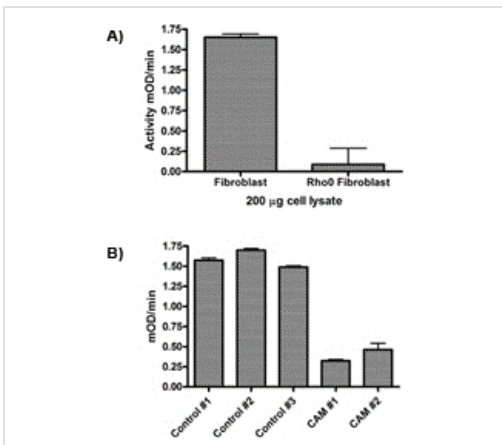


Mitochondrial ROS dependent functional deficiency and structural impairment in cardiac mitochondria after sepsis.

Image courtesy of Yao X et al. PLoS One. 2015; 10(10): e0139416. doi: 10.1371/journal.pone.0139416.

Mitochondrial fractions from the heart tissue of Rats infected by *S. pneumoniae*, or given PBS sham control, were subjected to measurements of complex I-V activities. Complex I was measured with [ab109721](#) (top left), Complex II + III were measured using [ab109905](#) (top right), Complex IV was measured using [ab109911](#) (bottom left) and Complex V was measured using [ab109714](#) (bottom right).

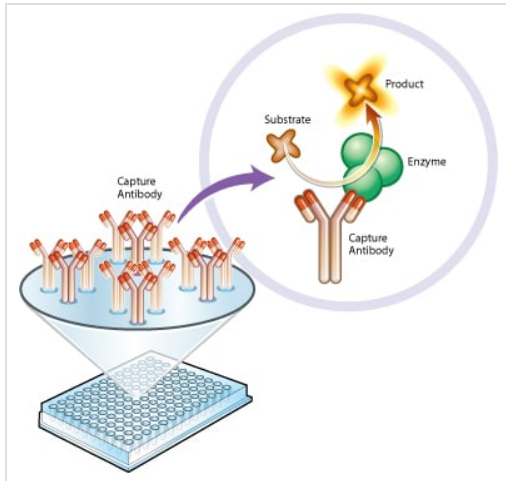
Freshly isolated mitochondrial pellets were resuspended in PBS supplemented with 10% detergent provided in the kits. Protein concentrations of these mitochondrial lysates were estimated and 25 µg (for complex I, IV and V) or 100 µg (for complex II+III) mitochondrial protein was used per reaction. Enzyme activities were measured spectrophotometrically in triplicate and expressed as changes of absorbance per minute per mg protein.



(A) [ab109721](#) was used to measure Complex I activity in normal and Rho0 human fibroblast whole cell lysates at 1 mg/mL. Rho0 cells are cells in which the mitochondrial DNA has been removed and therefore essential Complex I proteins are not expressed. As shown, the rho0 cells showed no/little complex I activity.

(B) In a similar analysis, rat cardiomyocytes were grown for 5 days in ± 40 µM chloramphenicol (CAM) to inhibit mitochondrial protein synthesis, Complex I assembly and hence activity was greatly reduced in samples loaded at 0.5 mg/mL whole cell lysates.

Functional Studies - Complex I Enzyme Activity
Microplate Assay Kit (ab109721)



Principle of Complex I Enzyme Activity Microplate Assay Kit (ab109721)

Functional Studies - Complex I Enzyme Activity
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