abcam

Product datasheet

Complex IV Rodent Enzyme Activity Microplate Assay Kit ab109911

**** 3 Abreviews 84 References 3 Images

Overview

Notes

Product name Complex IV Rodent Enzyme Activity Microplate Assay Kit

Detection methodColorimetric

Sample type Cell culture extracts, Tissue

Assay type Enzyme activity (quantitative)

Assay time 6h 00m

Species reactivity Reacts with: Mouse, Rat

Product overview Complex IV Rodent Enzyme Activity Microplate Assay Kit (ab109911) is used to determine the

activity of cytochrome c oxidase in a mouse sample with speed and simplicity. The COX enzyme is immunocaptured within the wells of the microplate and activity is determined colorimetrically by following the oxidation of reduced cytochrome c by the absorbance change at 550 nm. Included in this kit for performance of the activity assay are buffer, detergent, substrate, and 96-well

microplate with monoclonal antibody pre-bound to the wells of the plate, allowing for a stream-

lined assay.

Complex IV assay protocol summary:

- add samples to wells to capture enzyme and incubate for 3 hrs
- wash wells
- add reaction mix
- analyze with microplate reader in kinetic mode for 120 min

Buffer, detergent, and microplate should be stored at 4°C, Reagent C (reduced cytochrome c)

should be stored at -80°C.

Range of complex IV / cytochrome c oxidase assay kits

Biochemical assay - ab239711

Immunocapture with biochemical assay (plate-based)*** - ab109911 (rodent) (this

kit) and <u>ab109909 (human)</u>

*** Most popular assay format

Immunocapture with biochemical assay (dipstick) - ab109878 (rodent) and ab109876 (human)

Immunocapture with biochemical assay and ELISA - ab109910 (human)

ELISA - ab179880 (human)

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Other related products

Review the <u>mitochondrial assay guide</u>, or the full <u>metabolism assay guide</u> 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

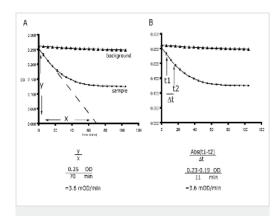
Properties

Storage instructions

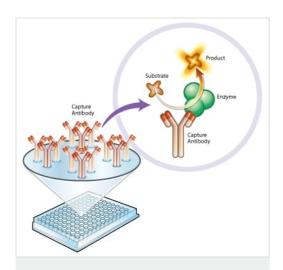
Please refer to protocols.

Components	96 tests
96-well microplate	1 unit
Tube 1 (Buffer)	1 x 10ml
Detergent	1 x 1ml
Reagent C (Reduced Cytochrome c)	1 x 1ml

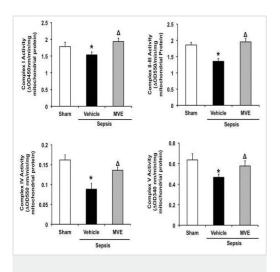
Images



Functional Studies - Complex IV Rodent Enzyme Activity Microplate Assay Kit (ab109911) Figure 1. To determine the activity in the sample, calculate the slope by using microplate software or by manual calculations using one of the two methods shown. Compare the sample rate with the rate of the control (normal) sample and with the rate of the null (background) to get the relative Complex IV activity. (A)The rate is determined by calculating the gradient of the initial slope over the linear region. (B) The rate is determined by calculating the slope between two points within the linear region.



Functional Studies - Complex IV Rodent Enzyme Activity Microplate Assay Kit (ab109911)



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.

Abcam's enzyme activity assays apply a novel approach, whereby target enzymes are first immunocaptured from tissue or cell samples before subsequent functional analysis. All of our ELISA kits utilize highly validated monoclonal antibodies and proprietary buffers, which are able to capture even very large enzyme complexes in their fully-intact, functionally-active states. Capture antibodies are pre-coated in the wells of premium Nunc MaxiSorp™ modular microplates, which can be broken into 8-well strips. After the target has been immobilized in the well, substrate is added, and enzyme activity is analyzed by measuring the change in absorbance of either the substrate or the product of the reaction (depending upon which enzyme is being analyzed). 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.

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

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

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