abcam

Product datasheet

Complex IV Human Enzyme Activity Dipstick Assay Kit ab109876

15 References 3 Images

Overview

Notes

Product name Complex IV Human Enzyme Activity Dipstick Assay Kit

Sample type Cell culture extracts, Tissue

Assay type Sandwich (quantitative)

Species reactivity Reacts with: Cow, Human

Does not react with: Mouse, Rat

Product overviewContains 30 or 90 dipsticks and necessary components to quantify the activity of the cytochrome

c oxidase enzyme complex from human and bovine samples. The kit includes sufficient materials

to generate a standard curve and evaluate several unknown samples.

The isolation of mitochondria is not necessary for the performance of this assay. In this kit the specificity of anti-COX monoclonal antibodies (mAbs) is combined with traditional methods for determining COX enzyme activity by histochemical methods and in-gel activity assays. First, the COX enzyme complex is immnocaptured (i.e immunoprecipated in active form) on the dipstick. Second, the dipstick is immersed in COX activity buffer containing reduced cytochrome c and diamino benzidinetetrachloride (DAB), which serves as the reporter of COX activity.

Immunocaptured COX oxidizes cytochrome c, which then oxidizes DAB to form a red-colored precipitate at the COX antibody line on the dipstick. In addition to being quick, the reaction is cyanide-sensitive. The signal intensity of this precipitate corresponds to the level of COX activity in the sample. The signal intensity is best measured by a dipstick reader or may be analyzed by

another imaging system.

Store dipsticks at room temperature in their provided container and out of direct sunlight. High

humidity conditions should be avoided.

Store Buffer A, B, and C at 4°C or at -20°C for long term storage.

Store Tubes 1 and 2 at -80 $^{\circ}$ C; they can also be aliquoted upon receipt to prevent freeze/thaw

cycles.

Tube 3 can be stored at room temperature.

Range of complex IV / cytochrome c oxidase assay kits

Biochemical assay - ab239711

1

Immunocapture with biochemical assay (plate-based) - ab109911 (rodent) and ab109909

(human)

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

(human) (this kit)

Immunocapture with biochemical assay and ELISA - ab109910 (human)

ELISA - ab179880 (human)

Tested applications

Suitable for: Functional Studies

Platform

Reagents

Properties

Storage instructions

Please refer to protocols.

Components	30 tests
96-well microplate	2 units
Buffer B (10X Blocking solution)	2 x 1ml
Dipsticks	1 x 30 units
Extraction Buffer (ab260490)	1 x 15ml
Tube 1 (DAB 100X stock)	1 x 100µl
Tube 2 (reduced cytochrome c 20X stock)	1 x 500µl
Tube 3 (Buffer for activity assay)	1 x 10ml
Wash buffer	1 x 1.5ml

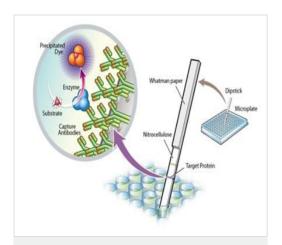
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab109876 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Functional Studies		Use at an assay dependent dilution.

Images



Functional Studies - Complex IV Human Enzyme Activity Dipstick Assay Kit (ab109876)

Abcam's enzyme activity assays apply a novel approach, whereby target enzymes are first immunocaptured from tissue or cell samples before subsequent functional analysis. Dipstick ELISA Kits extend this concept by utilizing the well-established lateral flow concept, wherein capture antibodies are striped onto nitrocellulose membrane and a wicking pad draws the sample through the antibody bands. 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.

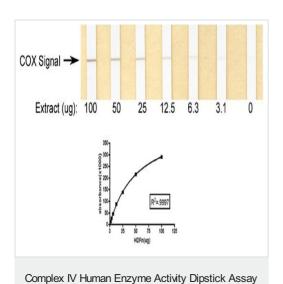
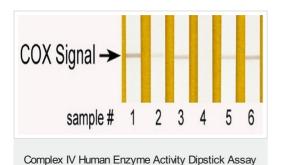


Figure 1. An example using ab109876 to measure Complex IV activity in fibroblast protein extracts. Developed dipsticks from a 1:2 dilution series using a positive control sample and the associated standard curve. Starting material was 100 μ g of fibroblast protein extract.



Kit (ab109876)

Kit (ab109876

Figure 2. An example using ab109876 to measure Complex IV activity in fibroblast protein extracts. Based on the standard curve, 50 μ g of protein extract were loaded onto a dipstick for each sample. The figure shows four developed dipsticks, a control sample (1) and four unknowns (2-6). The analysis of the signal intensity and interpolation from the standard curve showed that the unknown samples have between 15-61% of normal Complex IV activity levels.

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