

## Product datasheet

# Complex I Enzyme Activity Microplate Assay Kit (Colorimetric) ab109721

★★★★★ 4 Abreviews 72 References 5 Images

### Overview

<b>Product name</b>	Complex I Enzyme Activity Microplate Assay Kit (Colorimetric)
<b>Detection method</b>	Colorimetric
<b>Sample type</b>	Cell culture extracts, Tissue
<b>Assay type</b>	Enzyme activity
<b>Assay time</b>	3h 30m
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Cow, Human
<b>Product overview</b>	<p>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.</p> <p>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<sup>+</sup> 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.</p> <p>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.</p> <p>Note: This activity assay measures the diaphorase-type activity of Complex I. This activity is not dependant 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.</p> <p>Review our <a href="#">Metabolism Assay Guide</a> 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.</p>
<b>Notes</b>	ab109721 is shipped at 4°C. 20X NADH and 100X Dye are shipped lyophilized. Rehydrate 20X NADH by adding 1.1 mL H <sub>2</sub> O. Rehydrate 100X Dye by adding 0.25 mL H <sub>2</sub> O, 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

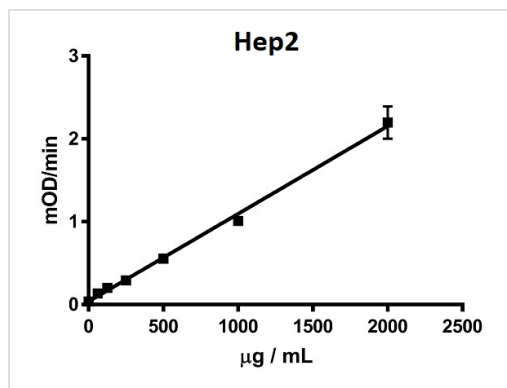
Microplate reader

### Properties

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

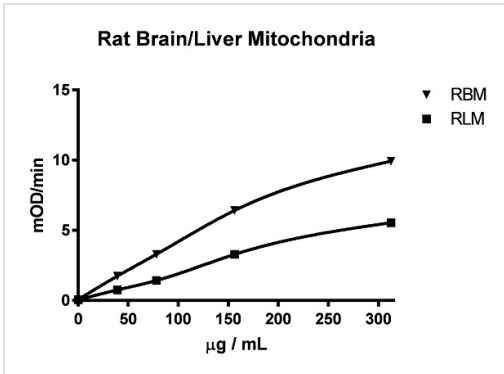
Components	96 tests
100X Dye (lyophilized)	1 unit
10X Blocking Solution	1 x 10ml
10X Detergent	1 x 1ml
20X Wash Buffer	1 x 25ml
20X NADH (lyophilized)	1 unit
96-well microplate (12 strips)	1 unit

### Images



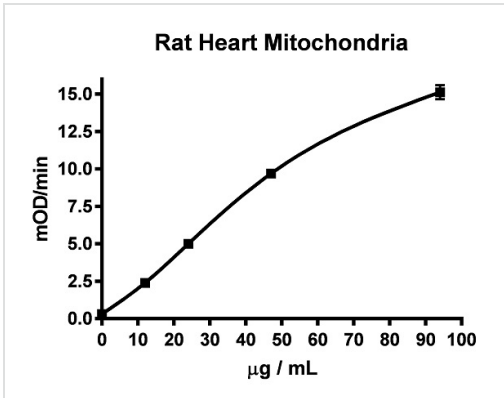
ab109721 measures Complex I activity in human cultured cells within the recommended ranges given in the protocol. Example of Complex I activity measured in Hep2 cells is shown. (Note that these ranges depend on mitochondria preparation quality).

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



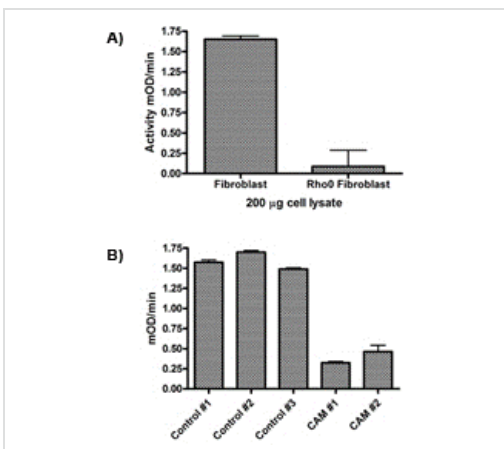
Functional Studies - Complex I Enzyme Activity  
Microplate Assay Kit (ab109721)

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).



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

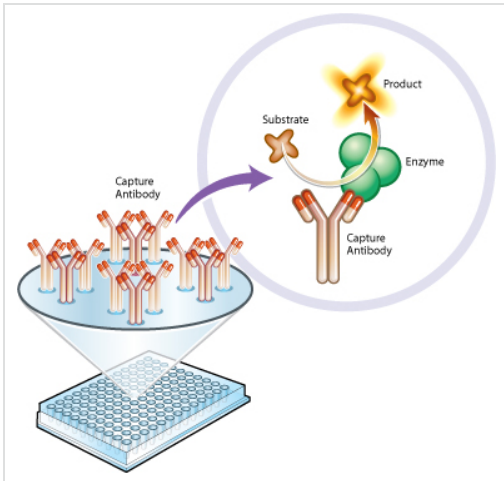
Examples of Complex I activity measured in different rat tissue mitochondrial samples are shown. (Note that these ranges depend on mitochondria preparation quality).



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

(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  $\pm 40 \mu\text{M}$  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.



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

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

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

### Our Abpromise to you: Quality guaranteed and expert technical support

---

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

### Terms and conditions

---

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors