Version 11 Last updated 25 January 2019

ab109721 Complex I Enzyme Activity Assay Kit (Colorimetric)

For the measurement of Complex I enzyme activity in human, rat, mouse and bovine mitochondria, cell and tissue extracts.

View kit datasheet: www.abcam.com/ab109721

(use www.abcam.cn/ab109721 for China, or www.abcam.co.jp/ab109721 for Japan)

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

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

Capture antibodies, pre-coated on the provided microplate, immobilize Complex I whose activity is determined by following the oxidation of NADH to NAD+ and the simultaneous reduction of the provided dye (ε = 25.9/mM/well) which leads to increased absorbance at OD 450 nm.

Please note that this activity assay measures the NADH-dependent 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, assembly deficiencies of Complex I can affect this activity assay.

2. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

Detergent extract of prepared samples loaded onto plate

Incubate for 3 hours at room temperature. Wash plate wells with Buffer 3X

Add 200 µL of Assay Solution to each well

Measure Optical Density (OD450 nm) in kinetic mode at room temperature for up to 30 minutes*

**For kinetic mode detection, assay measurement time is sample-specific and therefore suggestion is for guidance only.*

3. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt. Kit can be stored for 1 year from receipt, if components have not been reconstituted. Check storage of individual components after reconsitution.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
20X Wash Buffer	25 mL	4°C	4°C
10X Blocking Solution	10 mL	4°C	4°C
10X Detergent	1 mL	4°C	4°C
20X NADH (lyophilized) 40 mM after addition of H_2O	1 vial	4°C	-80°C
100X Dye (lyophilized)	1 vial	4°C	-80°C
96-well microplate (12 strips)	1 unit	4°C	4°C

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm.
- Ultra-pure water or double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Dounce homogenizer (if using tissue)
- Method for determining protein concentration: we recommend BCA Protein Quantification Kit (ab102536)
- For mitochondria isolation:
  Mitochondria Isolation Kit for Cultured Cells (ab110170)
  Mitochondria Isolation Kit for Tissue (ab110168) or
  Mitochondria Isolation Kit for Tissue (with Dounce Homogenizer) (ab110169)

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 20X Wash Buffer

Prepare 1X buffer by diluting 20X Wash Buffer in ddH2O: to make 500 mL 1X buffer, combine 25 mL 20X Wash Buffer with 475 mL ddH2O. Mix thoroughly and gently. Label this mixture as "1X Wash Buffer". Aliquot 20mL of this buffer and label it as "Dilution Buffer" to make activity buffer in step 8.3.1. Store Buffer at 4°C. Equilibrate to room temperature before use.

6.2 10X Blocking Solution

Dilute 10X Blocking Solution in 1X Wash Buffer to create Incubation Solution: to make 100 mL Incubation Buffer, combine 10 mL of 10X Blocking Solution with 90 mL of 1X Wash Buffer. Mix thoroughly and gently. Label this mixture as "Incubation Solution". Store Incubation Solution at 4°C. Equilibrate to room temperature before use.

- **Note**: 10X Blocking solution is also available as a separate product (ab126587)

6.3 20X NADH

Reconstitute the lyophilized NADH in 1.1 mL of ddH2O and mix thoroughly until dissolved to make a 40 mM solution. Aliquot reconstituted NADH so that you have enough volume to perform the desired number of assays. Store at -80°C. Reconstituted NADH is stable for up to 6 months. Keep on ice while in use.

6.4 100X Dye

Reconstitute the dye in 250 µL of ddH2O and mix thoroughly until dissolved. Aliquot reconstituted Dye so that you have enough volume to perform the desired number of assays. Reconstituted Dye is stable for up to 6 months. Store at -80°C. Keep on ice while in use.

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples. However, if you cannot perform the assay at the same time as the sample dilution and detergent extraction, we suggest that you freeze samples before extraction. Alternatively, if that is not possible, we suggest that you snap freeze detergent extracted samples in liquid nitrogen and store immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

Considering careful experimental design, inhibitors or activators of Complex I can be added to samples, for example cells in culture, before extraction or added directly to assay solution during enzyme activity analysis.

Sample preparation for three types of sample – cultured cells, tissue and mitochondria:

7.1 Preparation of extracts from cells (adherent or suspension):

1. Harvest suspension cells by centrifugation or scrape to collect adherent cells from a confluent culture flask (initial recommendation = 1 – 2 x 10^7 cells).
2. Wash cells twice with PBS.
3. Determine the sample protein concentration using a standard method such as BCA on a small portion of your sample. Then adjust concentration of the remaining sample with PBS so that the final sample protein concentration is 5.5 mg/mL.
   Alternatively, resuspend and dilute the cell pellet to the approximately required concentration (5.5 mg/mL) by simply adding 9 volumes of PBS (e.g. 50 µL pellet + 450 µL PBS to a total volume of 500 µL).

4. Using either method extract the proteins from the sample by adding 1/10 volume of 10X Detergent solution to sample (e.g. for a sample volume of 90 µL add 10 µL of 10X Detergent solution). Mix well by inversion.
   Δ **Note**: the sample concentration is now 5 mg/mL.
5. Incubate the tube on ice for 30 minutes to allow protein extraction.
6. Centrifuge the sample at 16,000 x g for 20 minutes at 4°C in a cold centrifuge.
7. Collect supernatant and transfer to a clean tube on ice.
8. Based on the protein concentration of sample extracted (5 mg/mL) dilute your samples to the desired concentration in Incubation Solution (from step 6.2). Table 1 indicates a typical linear range for the assay.

7.2 Preparation of extracts from tissue:

1. Harvest tissue for the assay (initial recommendation - 100 to 200 mg).
2. Wash tissue thoroughly in cold PBS.
3. Resuspend tissue in 500 µL – 1 mL of ice cold PBS.
4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 20 – 40 passes, or until sample is fully homogenized and is completely smooth.
5. Collect homogenate and transfer to a clean tube on ice.
6. Determine the sample protein concentration (using a standard method such as BCA) by extracting a portion of your sample. Adjust concentration of the sample with PBS so that the final sample protein concentration is 5.5 mg/mL.
7. Extract the proteins from the sample by adding 1/10 volume of 10X Detergent solution to sample (e.g. for a sample volume of 90 µL add 10 µL of 10X Detergent solution). Mix well.
8. Incubate the tube on ice for 30 minutes to allow solubilization.
9. Centrifuge the sample at 16,000 x g for 20 minutes at 4°C in a cold centrifuge.
10. Collect supernatant and transfer to a clean tube on ice.
    Δ **Note:** the sample concentration now is 5 mg/mL.
11. Dilute your samples to the desired concentration in Incubation Solution (from step 6.2). Table 1 indicates a typical linear range for the assay.

7.3 Preparation of extracts from mitochondria:

You can isolate mitochondria using mitochondrial isolation kits such Mitochondria Isolation Kit for Cultured Cells (ab110170) or Mitochondria Isolation Kit for Tissue (with Dounce Homogenizer) (ab110169).

1. Determine the sample protein concentration accurately using a standard method such as BCA on a small portion of your sample. Then adjust the concentration of the remaining sample with PBS to a final sample protein concentration of 5.5 mg/mL.
2. Extract the proteins from the sample by adding 1/10 volume of 10X Detergent solution to the sample (e.g. for a sample volume of 90 µL add 10 µL of 10X Detergent solution). Mix well by inversion.
   Δ Note: the sample concentration is now 5 mg/mL.
3. Incubate the tube on ice for 30 minutes to allow protein extraction.
4. Centrifuge the sample at 16,000 x g for 20 minutes at 4°C in a cold centrifuge.
5. Collect supernatant and transfer to a clean tube on ice.
6. Based on the protein concentration of sample extracted (5 mg/mL) dilute your samples to the desired concentration in Incubation Solution (from step 6.2). Table 1 indicates a typical linear range for the assay.

Sample Type	Recommended sample concentration (µg/mL)
Cell culture extracts	125 – 1250
Fibroblast (MRC5) cell extracts	500
Heptoblastoma (HepG2) cell extracts	1000
Tissue extracts (mitochondria)	25 – 500
Heart extracts	100
Liver extracts	250

Table 1. Typical ranges of measurement in µg/mL. 200 µL of sample is loaded per well.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay positive control samples, background control samples and experimental samples in duplicate or more.

8.1 Plate Loading

1. Add 200 µL of all samples to wells and record as a plate map.
2. Sample wells - add 200 µL of sample prepared as described in the Sample Preparation section to each well of the microplate that will be used for this experiment.
3. Recommend to include a user-defined sample as positive control or negative control.
4. Add 200µL incubation solution to wells and record as the background.
5. Incubate microplate for 3 hours at room temperature.

8.2 Washing

1. Wash wells by adding 300 µL of 1X Wash Buffer solution to each well used.
2. Empty the wells of the microplate by turning the plate over and shaking out any remaining liquid. Blot the plate face down on paper towel.
3. Repeat washing steps two more times. Do not aspirate the final wash until the assay solution is ready in 8.3.

8.3 Prepare Assay Solution

1. Prepare 1.75 mL of Assay Solution per strip used according to the table below.

Number of strips	1X Dilution Buffer (mL)	20X NADH (µL)	100X Dye (µL)	Total volume (mL)
1	1.645	87.5	17.5	1.75
2	3.29	175	35	3.5
3	4.935	262.5	52.5	5.25
4	6.58	350	70	7
5	8.225	437.5	87.5	8.75
6	9.87	525	105	10.5
7	11.515	612.5	122.5	12.25
8	13.16	700	140	14
9	14.805	787.5	157.5	15.75
10	16.45	875	175	17.5
11	18.095	962.5	192.5	19.25
12	19.74	1050	210	21

2. Empty the wells from the last wash in 8.2.
3. Add 200 µl of Assay Solution (from step 8.3.1) to each well carefully to avoid bubbles.
4. Any bubbles created should be popped rapidly. Place the plate in the reader and record with the settings below.
5. Save data, analyze as described in the "Data Analysis".

Mode	Kinetic
Wavelength:	450 nm
Time:	30 minutes
Interval:	20 sec - 1 min
Shaking:	Shake between readings
Temperature	Room temperature

Δ Note: Measuring Time can vary depending upon the enzyme activity in the samples.

9. Data Analysis

- Extinction coefficient for dye (ε) = 25.9/mM/well
- Final concentration of NADH is 2 mM.
- Complex I activity in each well is proportional to the increase in absorbance at OD 450 nm within each well. The activity is expressed as the change in absorbance per minute per amount of sample loaded into the well.
- If the sample background control is significant, then subtract the sample background control from the sample reading.
- Examine the linear rate of increase in absorbance at OD 450 nm over time. An example is shown below where the rate/slope is calculated between these time points. Most microplate software of performing this function. Repeat this for all samples.

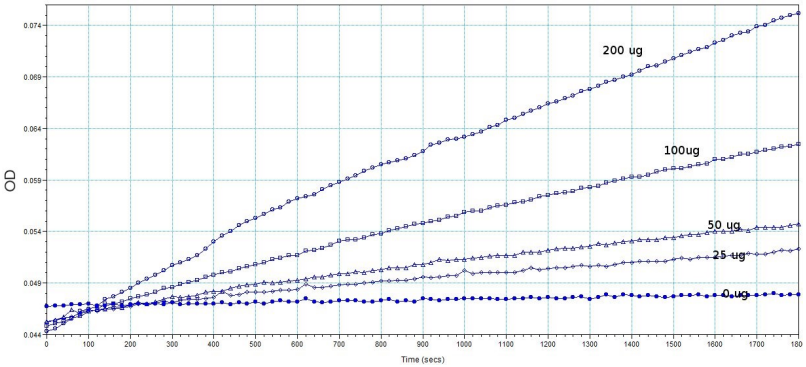
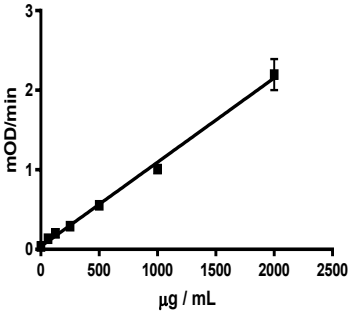


Figure 1. Example of raw data: change in OD 450 nm observed in cultured HepG2 cell lysate over time.



Raw data (as seen in Figure 1) can be expressed as rate (mOD/min) per µg/mL of cell lysate as shown below in Figure 2.

Figure 2. Complex I activity measured in cultured HepG2 cell lysate extract.

Precision

	Intra-Assay	Inter-Assay
n =	60	60
CV(%)	<10	<15

10. Typical Data

Data provided for **demonstration purposes only**.

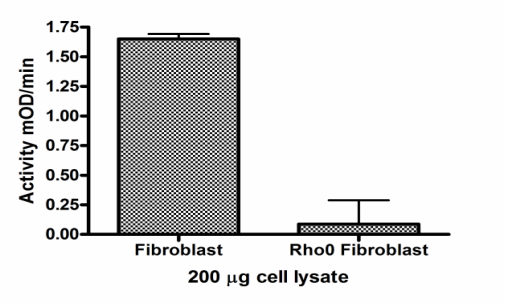
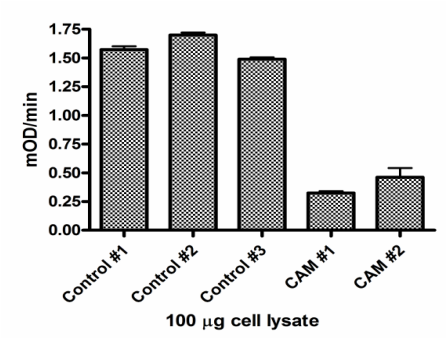


Figure 3. Complex I activity measured in normal and Rho0 human fibroblasts at 1mg/mL. Rho0 cells are cells in which the mitochondrial DNA has been removed and therefore essential Complex I proteins are not expressed. As shown in the right column, the rho0 cells showed no/little complex I activity.



40µM CAM	-	-	-	+	+

Figure 4. Complex I activity measured in rat cardiomyocytes (0.5 mg/mL) grown for 5 days in absence or presence of 40 µM chloramphenicol (CAM) to inhibit mitochondrial protein synthesis. In this case, both Complex I assembly and activity will be greatly reduced.

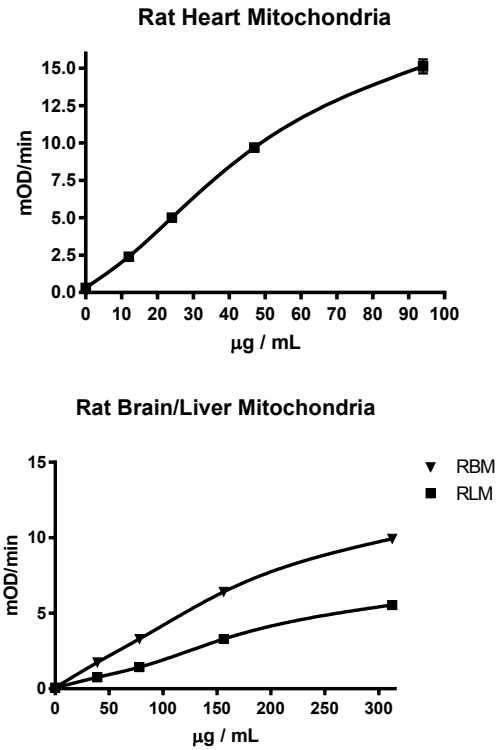


Figure 5. ab109721 measures Complex I activity in rat tissue mitochondria samples in the recommended ranges.

Δ Note: these ranges depend on mitochondria preparation quality.

11. FAQs / Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings or no signal	Samples not efficiently extracted with detergent	Ensure sufficient protein and adequate detergent to protein ratio as detailed in Section 7
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple freeze/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) until use
Lower/ Higher readings in samples and	Improperly thawed components	Thaw all components completely and mix gently

Standards		before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes, rapidly pop any visible bubbles using a needle
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

12. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | +400 921 0189 / 86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829