Mitochondrial Complex I Activity Colorimetric Assay

Introduction

Mitochondrial Complex I is the initiator and key rate-limiting enzyme of the respiratory chain, responsible for transferring electrons from NADH to ubiquinone, while driving proton transmembrane transport and providing the basis for ATP synthesis. Its dysfunction is closely related to various pathological processes such as neurodegenerative diseases, metabolic abnormalities, and aging. Therefore, accurate detection of complex I activity is of great significance for basic research and drug development.

Mitochondrial Complex I Activity Colorimetric Assay provides a fast and reliable method for determining the activity of mitochondrial complex I in purified mitochondria. It can be used to assess the effects of toxicants, drugs, and environmental factors on complex I function. This kit uses decylubiquinone as an electron acceptor, which is catalyzed by complex I to produce decylubiquinol. The latter then transfers electrons to Complex I Dye that absorbs light at 600 nm. Reduction of the dye results in a decrease in absorbance at 600 nm, allowing complex I activity to be quantitatively determined by monitoring this change. The kit also provides a complex I inhibitor, enabling the determination of specific mitochondrial complex I activity after subtracting the activity in presence of Complex I inhibitor from total activity.

Components and Storage

Components \ Size	100 Assays	Storage
Complex I Assay Buffer	25 mL	-20°C
100X NADH Stock	1 vial	-20°C away from light
Decylubiquinone	1 vial	-20°C away from light
Complex I Dye	1 vial	-20°C away from light
Complex I Inhibitor	100 µL	-20°C away from light
Shipping: Blue ice	**Shelf life: 1 year**	

Protocol

1. Preparation before the experiment

   1) 100X NADH stock preparation: Reconstitute the 100X NADH in 72 µL of ddH_2O to prepare a 100X NADH stock solution. Mix thoroughly. Aliquot and store unused stock solution at -20°C, protected from light.

2) 2X Decylubiquinone Stock Solution: Reconstitute Decylubiquinone in 350 µL of anhydrous ethanol to prepare a 2X Decylubiquinone Stock Solution. Mix thoroughly. Aliquot and store unused stock solution at -20°C in the dark. Anhydrous ethanol is volatile, ensure the tube is tightly sealed during storage.

3) 10X Complex I Dye Preparation: Reconstitute Complex I Dye well in 860 µL of Complex I Assay Buffer to prepare a 10X Complex I Dye (10 mM). Mix thoroughly. Aliquot and store unused solution at -20°C, protected from light.

***Note:** The prepared stock solutions are stable for at least 3 months when stored at -20°C.

2. Mitochondrial extraction

1) Extract mitochondria from cells or tissues using preferring protocol.

***Note:** Cell Mitochondria Isolation Kit I (Cat. No. K2724) and Tissue Mitochondria Isolation Kit (Cat. No. K2725) are recommended for extracting mitochondria from cells or tissues.

2) Determine the protein concentration of the isolated mitochondrial suspension. The mitochondrial protein concentration should be at least 500 µg/mL.

***Note:** The BCA Protein Assay Kit (Cat. No. K4101) is recommended for protein quantification.

3) Keep extracted mitochondria on ice if used immediately. For long-term storage, aliquot and store at -80°C.

3. Standard curve preparation

1) Dilute 10X Complex I Dye (10 mM) 1:10 with Complex I Assay Buffer to obtain 1X Complex I Dye (1 mM).

2) In a 96-well plate, pipette 0, 4, 8, 12, 16, and 20 µL of the 1X Complex I Dye (1 mM) into a series of wells to generate 0, 4, 8, 12, 16, and 20 nmol of dye per well.

3) Add the Complex I Assay Buffer to a final volume of 100 µL per well. Mix well.

4) Measure the absorbance at 600 nm.

4. Activity assay

1) Dilute the 2X Decylubiquinone in a 1:1 ratio with anhydrous ethanol to obtain 1X Decylubiquinone.

2) Refer to the table below to prepare each set in a 96-well plate and mix well.

	Blank control	Sample	Inhibitor control
Complex I Assay Buffer	59 µL	57 µL	56 µL
1X Decylubiquinone	2 µL	2 µL	2 µL
1X Complex I Dye (1 mM)	9 µL	9 µL	9 µL
Complex I Inhibitor	-	-	1 µL

3) For the sample and inhibitor control, add 2 µL of the extracted mitochondria (1-5 µg) per well and mix well.

*Note: Dilute the mitochondrial sample with Complex I Assay Buffer if the concentration is too high.

4) Dilute the 100X NADH stock solution 1:100 with Complex I Assay Buffer to obtain a 1X NADH working solution. Keep the 1X NADH on ice.

5) Using a multi-channel pipette, add 30 µL of the ice-cold 1X NADH working solution to each well. Immediately measure the absorbance at 600 nm.

*Note: It is recommended to use kinetic mode for detection, taking readings every 30 s for 5 min.

5. Activity calculation

1) First, establish the standard curve. Use the standard curve to obtain the amount of oxidized complex I dye in the sample.

2) Subtract the amount of oxidized Complex I Dye from the total Complex I Dye added in the assay (9 nmol/well) to obtain the amount of reduced Complex I Dye in the sample.

3) Apply the following formula to calculate Complex I activity.

Complex I Activity = ΔReduced Complex I dye concentration ÷ (Δt × p × d) (mUnits/µg)

ΔReduced Complex I dye concentration = change in reduced Complex I dye concentration during Δt

$\Delta t=T_2-T_1$ (min)

p = mitochondrial protein (µg)

d = sample dilution factor

4) Net Complex I activity in sample = activity in sample without inhibitor - activity in sample with inhibitor.

Unit definition: one unit of Complex I is the amount of enzyme that reduces 1.0 µmol of dye per minute at pH 7.4 at room temperature.

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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