Mitochondrial complex I Activity Assay Kit (colorimetric)

Cat #: orb1173204 (manual)

Product name: Mitochondrial complex I Activity Assay Kit (colorimetric)

Catalog number: orb1173204

Applicable samples: Animal and Plant Tissues, Cells

Storage: Stored at -20℃ for 6 months, protected from light

Assay Principle

Mitochondrial respiratory chain complex I (EC 1.6.5. 3), also known as NADH-CoQ reductase or NADH dehydrogenase, is the largest protein complex in the mitochondrial intima. Complex I is widely found in the mitochondria of animals, plants, microorganisms and cultured cells. This enzyme catalyzes the transfer of a pair of electrons from NADH to CoQ, and at the same time it can reduce O2 to produce O^{2-}, which is the main part of the respiration electron transport chain to produce O^{2-}. The activity of Complex I can not only reflect the status of responsibility electron transfer chain (ETC), but also reflect the production of reactive oxygen species (ROS). CheKine Micro Mitochondrial Complex I Activity Assay Kit provides a convenient tool for detection of Mitochondrial complex I Activity. The principle is that Complex I can catalyze the dehydrogenation of NADH to NAD^{+}.

The oxidation rate of NADH can be determined at 340 nm to calculate the activity of Complex I. It can be used to determine animal, plant tissue and cell samples.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	60 mL	60 mL×2	4℃
Reagent II	12 mL	24 mL	4℃
Reagent III	1 mL	2 mL	4℃, protected from light
Reagent IV	12.5 mL	25 mL	-20℃, protected from light
Reagent V	0.5 mL	1 mL	-20℃, protected from light
Reagent VI	Powder×1 vial	Powder×1 vial	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- Incubator, ice maker, refrigerated centrifuge

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- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer or mortar

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Working Reagent VI: Before use, for 48 T add 1 mL deionized water to dissolve it, for 96 T add 2mL deionized water to dissolve it. Please aliquot the unused reagents and store at -20℃, protected from light for 1 month. Avoid freezing and thawing.

Working Solution: Before use, Reagent IV and Reagent V were mixed at 99:1, and freshly prepared according to the dosage. Then incubated the mixture at 37℃ for 5 min if the detected samples are from mammalian, or incubated at 25℃ for 5 min if the samples are from another species.

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Note: Reagent IV is toxic, Reagent V has pungent odor, it is recommended to conduct experiments in the fume hood.

Sample Preparation

Note: Fresh samples are recommended to ensure enzyme activity.

Extraction of mitochondrial respiratory chain complex I:

1. Accurately weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Reagent I and 10 µL Reagent III, homogenize or mortar on ice.
2. Centrifuge the homogenate with 600 g for 5 min at 4℃, collect the supernatant to a new centrifuge tube and discard the pellet.
3. Centrifuge the supernatant again with 11,000 g for 10 min at 4℃. The pellet is the extracted mitochondria, which could be used to do step 5.
4. (Optional) The supernatant is cytoplasmic extract, which can be used as sample to determine mitochondrial respiratory chain complex I leaking from mitochondria to judge the effect of mitochondrial extraction.
5. Add 200 µL Reagent II and 2 µL Reagent III to the pellet, resuspend the pellet sufficiently, and use it to detect the activity of mitochondrial respiratory chain complex I in the next step.

Assay Procedure

1. Preheated the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Ultraviolet Spectrophotometer was returned to zero with deionized water.

2. Add 10 µL of sample, 200 µL of Working Solution and 15 µL of Working Reagent VI to the 96-well UV plate or microquartz cuvette, then tap the plate and mix well. Immediately read the initial 340 nm absorbance value A_1 at 0 min, and then read the absorbance value A_2 after 2 min, and calculate $\Delta A=A_1-A_2$.

Note: (1) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 1-2 samples. If the absorbance values is too high (above 1.5) or ΔA is greater than 0.4, the samples should be dilute with Reagent II and then measured again. Pay attention to multiply by the dilution factor when calculating the result. If ΔA is too small, the sensitivity can be improved by increasing the sample volume added. If ΔA is negative, it means that complex I is not contained in the sample or has been degraded. (2) The mitochondrial respiratory chain kit is based on the principle of enzyme kinetics, and the reaction is relatively fast, and after the reaction tends to balance, the reversible reaction may have a negative reaction. The suggestions are as follows: 1. The number of sample groups: about 2-3, the enzymatic reaction speed is fast, and it is an enzymatic reaction, it is necessary to grasp the starting time point and the time point after the reaction; 2. The instrument is preheated in advance, and the sample addition can be arranged next to the enzyme marker, and the sample is added directly after mixing; 3. If ΔA is too small, the sample size (tissue weight or cell number) can be increased, or the amount of extraction liquid can be reduced; 4. The samples should be extracted as fresh as possible. If they cannot be used immediately, the whole cells or packaged tissues should be stored at -80°C for use within one month. 5. Preparation of Working Solution before use. 6.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculated by fresh weight of samples

Unit definition: one enzyme activity unit defines as the consumption of 1 nmol NADH in 1 g tissue reaction system per min. Calculate the activity of complex I in the supernatant:

The activity of Complex I (U/g fresh weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Extracttion}\times V_{Sample})\div T$**=3,654×$\Delta A_{Supernatant}$÷W** Calculate the activity of complex I of the mitochondrial pellet

The activity of Complex I (U/g fresh weight) = $[\Delta A_{Pellet}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Resuspended}\times V_{Sample})\div T$**=731×$\Delta A_{Pellet}$÷W** Calculate the total activity of complex I in sample:

The total activity of complex I in sample is the sum of the activity of complex I in the supernatant and in the pellet. Calculated by fresh weight of samples:

Total activity (U/g fresh weight)**=3,654×$\Delta A_{Supernatant}$÷W+731×ΔA_{Pellet}÷W**

2. Calculated by cell density

Unit definition: Every 10,000 cells consume 1 nmol NADH per minute is defined as one unit of enzyme activity.

The activity of Complex I ($U/10^4$cell)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Resuspended}\times 500)\div T$**=1.46×ΔA**

Where: V_{Total}: total reaction volume, 2.25×10^{-4} L; ε: NADH molar extinction coefficient, 6.22×10^{3} L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^{9}: Unit conversion factor, 1 mol=10^{9} nmol; V_{Sample}: sample volume added, 0.01 mL; T: reaction time, 2 min; $\Delta A_{Supernatant}$: determination value of the supernatant; W:sample weight, g; $V_{Extracttion}$: sample extract volume, 1.01 mL; ΔA_{Pellet}: determination value of Pellet; $V_{Resuspended}$: Volume of the resuspend pellet 0.202 mL; 500: Total number of or cells, 5×10^{6}.

B. Microquartz cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Typical Data

Examples:

1. Test 0.1g cabbage, prepared the sample following the above protocol and measured with the 96-well UV microplate: $\Delta A_{Supernatant}=A_1-A_2=0.7819-0.7804=0.0015$. $\Delta A_{Pellet}=A_1-A_2=0.783-0.7704=0.0126$.
2. Calculated by fresh weight of samples

Calculated by fresh weight of samples in supernatant:

Complex I activity (U/g fresh weight)=$3,654\times\Delta A_{Supernatant}\div W$

=$3,654\times0.0015\div0.1=54.81$ U/g.

Calculated by fresh weight of samples in the pellet, Complex I activity (U/g fresh weight)=$731\times\Delta A_{Pellet}\div W$ =$731\times0.0126\div0.1=92.106$ U/g.

The total Complex I activity (U/g fresh weight)=$3,654\times\Delta A_{Supernatant}\div W+731\times\Delta A_{Pellet}\div W=54.81+92.106=146.916$ U/g.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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