PRODUCT INFORMATION & MANUAL

Mitochondrial Complex IV Activity Assay Kit (Colorimetric)

NBP3-25855

For research use only.
Not for diagnostic or therapeutic procedures.

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Mitochondrial Complex IV(Cytochrome C Oxidase)Activity Assay Kit

Catalog No: NBP3-25855

Method: Colorimetric method

Specification: 96T (Can detect 94 samples without duplication)

Instrument: Microplate reader

Sensitivity: 3.0 U/L

Detection range: 3.0–88.6 U/L

Average intra-assay CV (%): 5

Average inter-assay CV (%): 9.5

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure mitochondrial complex IV(cytochrome C oxidase)activity in animal tissue samples.

▲Detection principle

Mitochondrial complex IV, also known as cytochrome C oxidase, is one of the major enzymes in the mitochondrial respiratory chain. It oxidizes the reduced cytochrome C converted from mitochondrial complex III to oxidized cytochrome C and consumes oxygen to generate water. Mitochondrial complex IV can catalyze the oxidation of reduced cytochrome C to oxidized cytochrome C, which has an absorption wavelength at 550 nm. Therefore, the activity of mitochondrial complex IV can be quantified by measure the change OD value at 550 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution A	50 mL × 2 vials	-20℃ , 12 months
Reagent 2	Extraction Solution B	50 mL × 1 vial	-20℃ , 12 months
Reagent 3	Inhibitor	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Substrate	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 5	Stabilizer	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 6	Buffer Solution	26 mL× 1 vial	-20℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (540-560 nm, optimum wavelength: 550 nm), Centrifuge.

Reagents:

Anhydrous ethanol, PBS(0.01 M, pH 7.4).

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. It is recommended that the number of samples for an experiment be controlled within 5 samples.
2. The average ΔA value of blank well should be within ± 0.005.

Pre-assay preparation

▲ Reagent preparation

1. Bring reagent 1, reagent 2 and reagent 6 to room temperature before use, preserve reagent 3, reagent 4 and reagent 5 on ice for use.
2. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 with 1 mL anhydrous ethanol and mix fully. The prepared solution can be stored sealed at -20°C with shading light for 1 month.
3. Preparation of reagent 4 working solution:
   Dissolve a vial of reagent 4 with 4 mL reagent 6 and mix fully. Aliquot the prepared solution into small quantities and it can be stored at -20°C for 1 month. with shading light, avoid repeated freezing and thawin.
4. Preparation of reagent 5 working solution:
   Dissolve a vial of reagent 5 with 200 μL reagent 6 and mix fully. The prepared solution can be stored at -20°C for 1 month with shading light, avoid repeated freezing and thawing.
5. Preparation of reaction working solution:
   Mix the reagent 4 working solution and reagent 5 working solution at the ratio of 100:3 fully. Prepare the fresh needed amount before use and the prepared reaction working solution should be placed at room temperature with shading light for 10 min before use.

▲ Sample preparation

Tissue sample:

Weigh the tissue accurately, adding 9 times of the volume of reagent 1 according to the ratio of weight (g): volume (mL) =1:9. Then centrifuge at 600 g for 5 min at 4°C, discard the precipitate and take the supernatant. Then centrifuge at 11000 g for 10 min at 4°C. Discard the supernatant and take the precipitate, the precipitate was mixed with 200 μL of reagent 2 and 10 μL of reagent 3 working solution fully, sonicated for 1 min at 4°C, centrifuged at 11000×g at 4°C for 10 min. Then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (3.0–88.6 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse spleen tissue homogenate	1

Note: The diluent is double distilled water.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
B	S1	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
C	S2	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
D	S3	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S4	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S5	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S6	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
H	S7	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94

Note: A, blank wells; S1-S94, sample wells.

▲Detailed operation steps

(1) Blank well: Add 30 μL of regent 2 to blank well.

Sample well: Add 30 μL of sample to sample well.

(2) Add 120 μL of regent 6 to each well and mix fully with microplate reader for 3 s.

(3) Add 70 μL of reaction working solution to each well.

(4) Measure the OD value of each well at 550 nm with microplate reader at 10s and 70s, respectively recorded as A_1 and A_2, $\Delta A = A_1 - A_2$.

Note:The average ΔA value of blank well should be within ± 0.005.

▲Summary operation table

	Blank well	Sample well
Regent 2 (μL)	30	
Sample (μL)		30
Regent 6 (μL)	120	120
Mix fully with microplate reader for 3 s.		
Reaction working solution (μL)	70	70
Measure the OD value of each well at 10 s and 70 s, respectively recorded as A_1 and A_2, $\Delta A = A_1 - A_2$		

Note: The average ΔA value of blank well should be within ± 0.005.

▲Calculation

For tissue sample:

Definition: The amount of mitochondrial complex IV in 1 g tissue mitochondrial protein per 1 minute that oxidize 1 μmol of cytochrome C at room temperature is defined as 1 unit.

$$\text{mitochondrial complex IV activity (U/gprot)}$$

$$= \Delta A_{550} \times V_1 \div (V_2 \times \varepsilon \times d \times T) \div C_{pr} \times f$$

Note:

ΔA_{550}: $\Delta A_{sample} - \Delta A_{blank.}$

V_1: The volume of the reaction system, 0.22 mL.

V_2: The volume of the sample, 0.03 mL.

ε: Molar absorption coefficient, 0.0191 L/μmol/cm.

d: Optical path, 0.65 cm

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

C_{pr}: The concentration of protein in sample, gprot/L.

Appendix I Data

▲Example analysis

For rat liver tissue, take 30 μL of 10% rat liver tissue mitochondrial supernatant, and carry the assay according to the operation table.

The results are as follows:

the OD value of the sample A_1 is 0.458, the OD value of the sample A_2 is 0.402, the OD value of the blank A_1 is 0.394, the OD value of the blank A_2 is 0.393, the concentration of protein in sample is 3.32 gprot/L, and the calculation result is:

mitochondrial complex IV (U/gprot) = (0.458 - 0.402) - (0.394 - 0.393) × 0.22 ÷ (0.03 × 0.0191 × 0.65 × 1) ÷ 3.32 = 9.79 U/gprot