PRODUCT INFORMATION & MANUAL

Mitochondrial Complex II Activity Assay Kit (Colorimetric)

NBP3-25854

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

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Mitochondrial Complex II Activity Assay Kit

Catalog No: NBP3-25854

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.54 U/L

Detection range: 0.54-17.66 U/L

Average intra-assay CV (%): 4

Average inter-assay CV (%): 5

Average recovery rate (%): 97

▲ 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 II activity in animal tissue and cell samples.

▲Detection principle

Mitochondrial complex II, also known as succinate-coenzyme Q reductase, is widely found in the mitochondria of animals, plants, microorganisms and cultured cells. It catalyzes the oxidation of succinic acid in the TCA cycle to fumaric acid, which converts the ubiquinone to a reduced form through the basic unit structures of the complex such as iron-sulfur proteins. Coenzyme Q, the catalytic product of mitochondrial complex II, can further reduce 2, 6-dichloroindoxol, which has a characteristic absorption peak at 600 nm. Therefore, the activity of mitochondrial complex II can be quantified by measure the change OD value at 600 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution A	50 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Extraction Solution B	30 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Inhibitor	0.8 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 4	Buffer Solution	20 mL×1 vial	2-8℃ , 12 months
Reagent 5	Substrate A	1.6 mL×1 vial	2-8℃ , 12 months, shading light
Reagent 6	Substrate B	1.5 mL×2 vials	2-8℃ , 12 months, shading light
Reagent 7	Substrate C	1.2 mL×1 vial	2-8℃ , 12 months, shading light
	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 (590-610 nm, optimum wavelength: 600 nm), Centrifuge

Reagents:

Normal saline (0.9% NaCl), 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. When adding the reaction working solution, adhere to the wall and add slowly to avoid bubbles..
2. Use fresh samples for detection, processed samples should be tested the same day.
3. It is recommended that the number of samples for an experiment be controlled within 10 samples.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reaction working solution:

   Mix the reagent 4, reagent 5, reagent 6 and reagent 7 at the ratio of 15:1:2:1 fully, incubate at 37°C for 10 min. Prepare the fresh needed amount before use and the prepared solution should be store with shading light and used within 1 day.

▲ Sample preparation

1. Tissue sample:

Accurately 0.1 g weigh the tissue, then add 0.9 mL reagent 1 to homogenize the sample. Then centrifuge at 600 g for 5 min at 4°C, discard the precipitate and take the supernatant. Then centrifuge at 15000 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, sonicated for 1 min at 4°C, centrifuged at 15000×g at 4°C for 10 min. Then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

2. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Mix 1×10^6 cells with 400 μL of reagent 1 fully and homogenize. Then centrifuge at 600 g for 5 min at 4°C, discard the precipitate and take the supernatant. Then centrifuge at 15000 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, sonicated for 1 min at 4°C, centrifuged at 15000×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 (0.54-17.66 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 heart tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
Jurkat cell	1

Note: The diluent is reagent 2.

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, Sample well: Add 190 μL of reaction working solution to each well.

(2) Incubate at 37°C for 3 min.

(3) Blank well: Add 20 μL of regent 2 to blank well.

Sample well: Add 20 μL of sample to sample well.

(4) Mix fully with microplate reader for 3 s and measure the OD value of each well at 600 nm with microplate reader, recorded as A_1. Incubate at 37°C for 3 min, measure the OD value of each well at 600 nm with microplate reader,, recorded as A_2, $\Delta A = A_1 - A_2$.

Note:When adding the reaction working solution, adhere to the wall and add slowly to avoid bubbles.

▲Summary operation table

	Blank well	Sample well
Reaction working solution (μL)	190	190
Incubate at 37°C for 3 min.		
Sample (μL)		20
Regent 2 (μL)	20	
Mix fully and measure the OD value of each well, recorded as A_1. Incubate at 37°C for 3 min, measure the OD value of each well, recorded as A_2, $\Delta A = A_1 - A_2$		

Note:When adding the reaction working solution, adhere to the wall and add slowly to avoid bubbles.

▲Calculation

1. For tissue and cell :

Definition: The amount of mitochondrial complex II in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol product at room temperature is defined as 1 unit.

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

$$= (\Delta A_{samlple} - \Delta A_{blank}) \times V_{total} \times f \div (V_{sample} \times 21.8^{*} \times T \times C_{pr}) \times 1000^{*}$$

Note:

ΔA_{sample}: The change OD value of sample (A_1 - A_2)

ΔA_{blank}: The change OD value of blank (A_1 - A_2).

f: Dilution factor of sample before test.

V_{total}: The volume of the reaction system, 0.21 mL.

V_{sample}: The volume of the sample, 0.02 mL.

21.8*: Molar absorption coefficient.

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

T: The time of reaction, 3 min.

1000*:1 mmol/L=1000 μmol/L.

Appendix I Data

▲Example analysis

For rat heart tissue, take 20 μL of 10% rat heart tissue homogenate, and carry the assay according to the operation table.

The results are as follows:

the OD value of the blank A_1 is 0.979, the OD value of the blank A_2 is 0.972, the OD value of the sample A_1 is 0.671, the OD value of the sample A_2 is 0.310, the concentration of protein in sample is 2.07gprot/L, and the calculation result is:

mitochondrial complex II activity (U/gprot) = (0.671 - 0.310) - (0.979 - 0.972) × 0.21 ÷ (0.02 × 21.8 × 3 × 2.07) × 1000 = 27.61 U/gprot