PRODUCT INFORMATION & MANUAL

Mitochondrial Complex III Activity Assay Kit (Colorimetric)

NBP3-25841

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

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Mitochondrial Complex III (Coenzyme Q-Cytochrome C Reductase) Activity Assay Kit

Catalog No: NBP3-25841

Method: Colorimetric method

Specification: 96T (Can detect 47 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 4.45 U/L

Detection range: 4.45–106.8 U/L

Average intra-assay CV (%): 5

Average inter-assay CV (%): 10

Average recovery rate (%): 100

- ▲ 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 III (Coenzyme Q-Cytochrome C Reductase) activity in animal tissue sample.

▲Detection principle

As an organelle, mitochondria is the power factory in cells and the main site of aerobic respiration of cells. Its function is to convert energy through oxidative phosphorylation to provide energy for cellular activities. The oxidation process is carried out by four respiratory chain membrane protein complexes (complexes I, II, III and IV) on the inner mitochondrial membrane. Mitochondrial complex III, also known as cytochrome c reductase complex, its main function is to oxidize the reduced coenzyme Q10 formed by mitochondrial complexes I and II to oxidative coenzyme Q10. In this process, the OD value increased at 550 nm. Therefore, the activity of mitochondrial complex III can be quantified by measure the change OD value at 550 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution A	55 mL × 2 vials	-20℃ , 12 months
Reagent 2	Extraction Solution B	26 mL × 1 vial	-20℃ , 12 months
Reagent 3	Extraction Solution C	1.2 mL × 2 vials	-20℃ , 12 months shading light
Reagent 4	Substrate A	6 mL× 1 vial	-20℃ , 12 months, shading light
Reagent 5	Diluent	14 mL× 1 vial	-20℃ , 12 months
Reagent 6	Substrate B	1.6 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 7	Stabilizer	Powder × 6 vials	-20℃ , 12 months, shading light
Reagent 8	Buffer Solution	26 mL× 1 vial	-20℃ , 12 months
Reagent 9	Inhibitor Controlled Solution	3 mL× 1 vial	-20℃ , 12 months, shading light
Reagent 10	Inhibitor	3 mL× 1 vial	-20℃ , 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

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

⚠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. The mitochondrial samples should be detected within 4 h as far as possible. If the samples are placed for a long time, the specific enzyme activity will be reduced, and the results of the sample determination will be low.
2. The detection is started at about 10 s after adding reagent 4, and it's better to measure no more than 4 samples at same time.
3. The change OD value of blank well should be within ± 0.005, indicating that the reagents are available. if it exceeds this range, it is necessary to check whether reagent 6 is clear and extend the incubation time.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 6 before use:

   Place reagent 6 at 37°C for 10 min before use, and mix the solution until its clarified for use. The reagent 6 can be divided into smaller packages at -20°C for 1 month.
3. Preparation of reagent 7 working solution:

   Dissolve a vial of reagent 7 with 2 mL reagent 5 and mix fully. Place the prepared solution on the ice box with shading light for use and the prepared solution should be used within 6 h.
4. Preparation of reaction working solution:

   Mix the reagent 7 working solution and reagent 6 at the ratio of 1:2 fully. Prepare the fresh needed amount before use. Stand the prepared solution at room temperature with shading light for 3 min, then use immediately. And the prepared solution should be used within 30 minutes.

▲Sample preparation

Tissue:

Weigh the tissue accurately, adding reagent 1 according to the ratio of weight (g): volume (mL) =1:9 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 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, sonicated for 1 min, centrifuged at 11000 g at 4°C for 10 min. Then take the supernatant for detection. Meanwhile, determine the mitochondria 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 (4.45–106.8 U/L).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	1-2
10% Rat kidney tissue homogenate	1-2
10% Rat brain tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1-2
10% Mouse kidney tissue homogenate	1-2
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse spleen tissue homogenate	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	X	X	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'
B	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
C	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
D	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
E	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
F	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
G	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
H	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'

[Note]: X, blank wells; S1–S47, total enzyme activity sample wells; S1'–S47', non–specific enzyme activity sample wells.

▲Detailed operation steps

1. Sample pretreatment

Total enzyme activity sample: Mix the sample and reagent 9 at the volume of 20 μL : 20 μL fully. Stand the prepared solution at room temperature with shading light for 5 min for use.

Non-specific enzyme activity sample: Mix the sample and reagent 10 at the volume of 20 μL : 20 μL fully. Stand the prepared solution at room temperature with shading light for 5 min for use.

2. The measurement of samples

(1) Blank well: Add 10 μL of reaction working solution to the corresponding wells.

Total enzyme activity sample well: Add 10 μL of reaction working solution to the corresponding wells.

Non-specific enzyme activity sample: Add 10 μL of reaction working solution to the corresponding wells.

(2) Add 90 μL of reagent 8 to each well.

(3) Blank well: Add 20 μL of reagent 2 to the corresponding wells.

Total enzyme activity sample well: Add 20 μL of total enzyme activity sample to the corresponding wells.

Non-specific enzyme activity sample: Add 20 μL of non-specific enzyme activity sample to the corresponding wells.

(4) Add 40 μL of reagent 4 to each well. Mix fully with microplate reader for 5 s.

(Because of the rapid enzymatic reaction, it is recommended to add reagent 4 using a multi-pipe pipettor, and it's better to measure no more than 4 samples at same time when using ordinary pipettor).

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

▲Summary operation table

	Blank well	Total enzyme activity sample well	Non-specific enzyme activity sample well
Reaction working solution (μL)	10	10	10
Reagent 8 (μL)	90	90	90
Reagent 2 (μL)	20		
Total enzyme activity sample (μL)		20	
Non-specific enzyme activity sample (μL)			20
Reagent 4 (μL)	40	40	40
Mix fully. Measure the OD value of each well at 10 s and 70 s respectively, recorded as A_1, A_2, $\Delta A = A_2 - A_1$.			

▲Calculation

Tissuel:

Definition: The amount of mitochondrial complex III in 1 g tissue mitochondria protein per 1 minute that reduce 1 μmol cytochrome c at room temperature is defined as 1 unit.

$$\text{mitochondrial complex III activity (U/gprot)} = (\Delta A_{Total} - \Delta A_{Non\text{-}specific}) \times V_1 \div V_2 \div (\varepsilon \times d) \div T \div C_{pr} \times f$$

Note:

ΔA_{Total}: The change OD value of total enzyme activity sample well ($A_2 - A_1$).

$\Delta A_{Non\text{-}specific}$: The change OD value of non-specific enzyme activity sample well ($A_2 - A_1$).

V_1: The volume of the reaction system, 0.16 mL.

V_2: The volume of the sample, 0.02 mL.

ε : The molar extinction coefficient of cytochrome c at 550 nm, 0.0191 L/μmol/cm

d: Optical path, 0.5 cm

T: The time of reaction, 1 min.

f: Dilution factor of sample before test.

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

Appendix I Data

▲Example analysis

For 10% rat kidney tissue mitochondria supernatant, dilute for 2 times, carry the assay according to the operation table.

The results are as follows:

The A_1 of the blank well is 0.198, the A_2 of the blank well is 0.199, ΔA = 0.001, indicating that the reagents are available. The A_1 of total enzyme activity sample well is 0.239, the A_2 of total enzyme activity sample well is 0.306, ΔA_{Total} = 0.067. The A_1 of non-specific enzyme activity sample well is 0.235, the A_2 of non-specific enzyme activity sample well is 0.289, $\Delta A_{Non\text{-}specific}$ = 0.054., the concentration of mitochondria protein in sample is 5.24 gprot/L, and the calculation result is

mitochondrial complex III activity (U/gprot)

= (0.067 – 0.054) × 0.16 ÷ 0.02 ÷ (0.01917 × 0.5) ÷ 1 ÷ 5.24 × 2

= 4.16 U/gprot