PRODUCT INFORMATION & MANUAL

Mitochondrial Complex V Activity Assay Kit (Colorimetric)

NBP3-25842

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

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Novus kits are guaranteed for 6 months from date of receipt

Mitochondrial Complex V (F_0F_1-ATPase/ATP Synthase) Activity Assay Kit

Catalog No: NBP3-25842

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Instrument: Microplate reader

Sensitivity: 12.13 U/L

Detection range: 12.13-74.21 U/L

Average intra-assay CV (%): 5.6

Average inter-assay CV (%): 6.5

Average recovery rate (%): 101

- 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 V (F_0F_1-ATPase/ATP Synthase) in animal tissue samples.

▲Detection principle

Mitochondrial complex V is also known as F_0F_1-ATP synthase. ATP is hydrolyzed by F_0F_1-ATP synthase to produce ADP, and ADP converts NADH into oxidized coenzyme I (NAD^+) after enzyme conversion reaction. Therefore, the activity of mitochondrial complex V can be quantified by measure the change OD value at 340 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution A	50 mL × 2 vials	-20°C , 12 months
Reagent 2	Extraction Solution B	50 mL × 1 vial	-20°C , 12 months
Reagent 3	Protease Inhibitor	0.8 mL × 2 vials	-20°C , 12 months, shading light
Reagent 4	Buffer Solution	30 mL×1 vial	-20°C , 12 months
Reagent 5	Substrate A	Liquid × 2 vials	-20°C , 12 months, shading light
Reagent 6	Substrate B	Powder × 2 vials	-20°C , 12 months, shading light
Reagent 7	Substrate C	Powder × 2 vials	-20°C , 12 months, shading light
Reagent 8	Substrate D	Powder × 2 vials	-20°C , 12 months, shading light
Reagent 9	Inhibitor	0.1 mL× 1 vial	-20°C , 12 months, shading light
	UV 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, 37 °C incubator, Microplate reader (330-350 nm, optimum wavelength: 340 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. During reagent preparation, it is necessary to ensure that the powder is completely dissolved in the reaction working solution after preparation.
2. The detection is started at about 10 s after adding reaction working solution.
3. For sample detection, if the A_1 of the sample well and the control well is lower than 0.7, or the change OD value (ΔA) of the sample well and the control well for 4 minutes exceeds 0.3, the sample should be diluted.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reaction working solution:
   Mix 15 mL reagent 4 with a vial of reagent 5, a vial of reagent 6 and a vial of reagent 7 in turn to ensure that the liquid or powder in each tube is fully dissolved. The prepared solution can be stored at 2-8°C with shading light for 8 h.
3. Preparation of enzyme working solution:
   Dissolve a vial of reagent 8 with 1.2 mL double distilled water and mix fully. Prepare the fresh needed amount before use. The prepared solution can be stored at 2-8°C with shading light for 8 h.
4. Preparation of specific working solution:
   Mix the reagent 9 and double distilled water at the ratio of 1:100 fully. Prepare the fresh needed amount before use and preserve it with shading light for use. The remaining reagent 9 can be stored at -20°C for 1 week.

▲ Sample preparation

1. Tissue sample:
   Accurately weigh 0.1g 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 5 min, 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. Collect 5×10^6 cells, add 900 ul of reagent 1 to 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 5 min, 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 (12.13-74.21 U/L).

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-6
10% Rat liver tissue homogenate	1-4
10% Mouse kidney tissue homogenate	2-6
10% Rat muscle tissue homogenate	1-3
5×10^6 HL-60 cells	1-2
5×10^6 Jurkat cells	1-2

Note: The diluent is reagent 2.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

Note: S1-S48: Sample wells; S1 '-S48' : Control wells.

▲Detailed operation steps

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

Sample well: Add 10 μL of double distilled water to the corresponding wells.

(2) Add 20 μL of sample to each well.

(3) Mix fully with microplate reader for 3 s. And incubate at 37°C for 4 min.

(4) Add 20 μL of enzyme working solution to each well.

(5) Add 180 μL of reaction working solution to each well.

(6) Measure the OD value of each well at 340 nm with microplate reader, recorded as A_1. After 4 min, measure the OD value of each well at 340 nm with microplate reader, recorded as A_2. $\Delta A = A_1 - A_2$. (It is recommended to follow the notes below for measurement).

Note:The sample wells measure the total enzyme activity, and the control wells measure the non-specific enzyme activity. After adding the reaction working solution, record the OD value once every minute for 4 min, observe the change of OD value within 4 min to ensure whether is a constant rate of decline. Otherwise, the sample needs to be diluted. When calculating, take initial OD value A_1, A_2 OD value after 4 min.

▲Summary operation table

	Control well	Sample well
Specific working solution (μL)	10	
Double distilled water (μL)		10
Sample (μL)	20	20
Mix fully. And incubate at 37°C for 4 min.		
Enzyme working solution (μL)	20	20
Reaction working solution (μL)	180	180
Measure the OD value of each well, recorded as A_1. After 4 min, measure the OD value of each well, recorded as A_2, $\Delta A = A_1 - A_2$. (It is recommended to follow the notes below the operation steps)		

Note:The sample wells measure the total enzyme activity, and the control wells measure the non-specific enzyme activity. After adding the reaction working solution, record the OD value once every minute for 4 min, observe the change of OD value within 4 min to ensure whether is a constant rate of decline. Otherwise, the sample needs to be diluted. When calculating, take initial OD value A_1, A_2 OD value after 4 min.

▲Calculation

For tissue and cell :

Definition: The amount of mitochondrial complex V in 1 g mitochondrial protein per 1 minute that catalyze decomposition of 1 μmol NADH at 37°C is defined as 1 unit.

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

$$= (\Delta A_{samlple} - \Delta A_{control}) \div (6220 \times 0.65) \times 0.23 \div t \div 0.02 \div C_{pr} \times f \times 10^6$$

Note:

ΔA_{sample}: The change OD value of sample, $A_1 - A_2$

$\Delta A_{control}$: The change OD value of control, $A_1 - A_2$

6220: Molar absorption coefficient, L/(μmol•cm)

0.65: Optical path, cm

0.23: The volume of the reaction system, mL.

0.02: The volume of the sample, mL.

T: The time of reaction, 4 min.

f: Dilution factor of sample before test.

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

10^6: 1 mol = 10^6 μmol.

Appendix I Data

▲Example analysis

For 10% mouse liver tissue mitochondria supernatant, dilute for 4 times, carry the assay according to the operation table. The results are as follows.

The results are as follows:

The A_1 of the sample well is 0.900, the A_2 of the sample well is 0.426, ΔA_{sample} = 0.900 - 0.426 = 0.474. The A_1 of control well is 0.914, the A_2 of control well is 0.471, $\Delta A_{control}$ = 0.914 - 0.471 = 0.443, the concentration of mitochondria protein in sample is 12.14 gprot/L, and the calculation result is:

mitochondrial complex V activity (U/gprot) = (0.474 - 0.443) ÷ (6220 × 0.65) × 0.23 ÷ 4 ÷ 0.02 ÷ 12.14 × 4 × 10^6 = 7.26 U/gprot