PRODUCT INFORMATION & MANUAL

Aconitase Activity Assay Kit (Colorimetric)

NBP3-25848

For research use only.

Not for diagnostic or therapeutic procedures.

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Aconitase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25848

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 2.32 U/L

Detection range: 2.32-60.19 U/L

Average intra-assay CV (%): 9.5

Average inter-assay CV (%): 5.9

Average recovery rate (%): 103

- ▲ 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 be used to measure aconitase (ACO) activity in animal tissue samples.

▲Detection principle

Aconitase (ACO) is an important Fe/S protein enzyme in cells, which mainly exists in cytoplasm and mitochondria. ACO catalyzes the reversible reaction of citric acid to isocitric acid from the intermediate product cisaconite acid in the cell, which plays an important role through maintaining the tricarboxylic acid cycle and glyoxylic acid cycle.

Aconitase catalyzes isocitrate to produce cis-aconitase. Cis-aconitase has a characteristic absorption peak at 240 nm. The activity of ACO was calculated by measuring the production rate of cis-aconitase.

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▲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	1.4 mL × 1 vial	-20°C , 12 months, shading light
Reagent 4	Buffer Solution	30 mL × 1 vial	-20°C , 12 months
Reagent 5	Substrate	0.15 mL × 4 vials	-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

Microplate reader (230-250 nm, optimum wavelength: 240 nm)

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 reagent 4, it should be added slowly by touching the wall to avoid bubbles.
2. It's recommended to measure no more than 10 sample wells at same time.
3. It is recommended to use fresh samples. Samples are easy to deactivate, so the prepared samples should be placed on the ice box, and it is recommended to use up within 2 hours.
4. Reagent 5 is prone to be oxidized and should be covered promptly after use.

Pre-assay preparation

▲ Reagent preparation

Bring all reagents to room temperature before use.

▲ Sample preparation

1. Tissue sample:

Accurately weigh the tissue of 0.1 g, add 0.9 mL of reagent 1 and 0.01mL of reagent 3. Homogenize the tissue sample with homogenizer on ice. Centrifuge the homogenized tissue at 600 g for 5 min at 4 °C , then take the supernatant and discard the precipitate. Then centrifuge the supernatant at 15000 g for 10 min at 4 °C and transfer the supernatant to another tube. The supernatant was used to determine the activity of ACO in cytoplasm. Meanwhile, determine the cytoplasmic protein concentration of supernatant. Take the precipitate, add 200 μL of reagent 2 and 2 μL of reagent 3, mix the reagent fully and sonicate for 3 min. Centrifuge the reagent at 15000 g for 10 min at 4 °C , then take the supernatant and discard the precipitate. The supernatant was used to determine the activity of ACO in mitochondria. Meanwhile, determine the mitochondria protein concentration of supernatant.

2. Cell sample:

Collect the 1×10^6 cells, add 0.4 mL of reagent 1 and 0.01mL of reagent 3. Homogenize the cells sample with homogenizer on ice. Centrifuge the homogenized cells at 600 g for 5 min at 4°C , then take the supernatant and discard the precipitate. Then centrifuge the supernatant at 15000 g for 10 min at 4°C and transfer the supernatant to another tube. The supernatant was used to determine the activity of ACO in cytoplasm. Meanwhile, determine the cytoplasmic protein concentration of supernatant.Take the precipitate, add 200 μL of reagent 2 and 2 μL of reagent 3, mix the reagent fully and sonicate for 1 min. Centrifuge the reagent at 15000 g for 10 min at 4°C ,then take the supernatant and discard the precipitate. The supernatant was used to determine the activity of ACO in mitochondria. Meanwhile, determine the mitochondria protein concentration of supernatant.

▲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 (2.32-60.19 U/L).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	6-10
10% Mouse kidney tissue homogenate	4-6
10% Mouse liver tissue homogenate	6-10
10% Mouse brain tissue homogenate	4-6
10% Rat lung tissue homogenate	6-10
10% Mouse muscle tissue homogenate	2-4

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	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

Note:S1-S96, sample wells.

▲Detailed operation steps

(1) Incubation of the sample: Mix the supernatant of sample and reagent 5 at volume ratio of 80: 1(the addition volume of reagent 5 is recommended to be more than 10 μL) fully. Incubate at 37 °C for 10 min, and preserve sample on ice for detection.

(2) Sample well: add 20 μL of sample and 180 μL of reagent 4 to each well.

(3) Mix fully with microplate reader for 3 s. Measure the OD value of each well at 240 nm with microplate reader, recorded as A_1. Incubate at 25°C for 4 min, measure the OD value of each well at 240 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.

▲Summary operation table

	Sample well
Incubation of the sample: Mix the supernatant of sample and reagent 5 at volume ratio of 80 : 1 (the addition volume of reagent 5 is recommended to be more than 10 μL) fully. Incubate at 37°C for 10 min, and preserve sample on ice for detection.	
Sample (μL)	20
Reagent 4 (μL)	180
Mix fully for 3 s. Measure the OD value of each well recorded as A_1. Incubate at 25°C for 4 min, measure the OD value of each well recorded as A_2. $\Delta A = A_2 - A_1$.	

▲Calculation

Tissue samples:

Definition: The enzyme in 1 mg mitochondria protein that catalyze the reaction to produce 1 nmol of cis-aconitic in 1 minute is defined as 1 unit.

$$\text{ACO activity (U/mgprot)} =$$

$$\Delta A \div (3.6 \times 0.6) \times 0.0002 \div T \div 0.02 \div C_{pr} \times f \times 10^6$$

Note:

ΔA: $A_2 - A_1$.

3.6: The molar absorption coefficient, L/mmol/cm.

0.6: Optical path, cm.

0.0002: The total volume of the reaction system, L.

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

T: The time of reaction, 4 min.

f: Dilution factor of sample before test.

0.02: The volume of sample, mL.

10^6:1 mmol = 1×10^6 nmol.

Appendix I Data

▲Example analysis

For rat liver tissue, dilute the mitochondria sample of 5% rat liver tissue homogenate for 4 times with reagent 2, take 20 uL of the diluted sample, and carry the assay according to the operation table.

The results are as follows:

the A_1 of the sample is 0.476, after 4 minutes of reaction, the A_2 of the sample is 0.546, $\Delta A = A_2 - A_1 = 0.546 - 0.476 = 0.07$; the protein concentration of the mitochondria sample is 9.04 mgprot/L, and the calculation result is:

$$\text{ACO activity (U/mgprot)} = (0.07 \times 0.0002 \times 4) \div (3.6 \times 0.6 \times 0.02 \times 9.04 \times 4) \times 10^6$$

$$= 35.85 \text{ U/mgprot}$$