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- Expressversand

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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K656-M

Specification：48T(48 samples)/96T(96 samples)

Measuring instrument：Microplate reader（230-250 nm）

Detection range：2.32-60.19 U/L

Elabscience®Aconitase(ACO) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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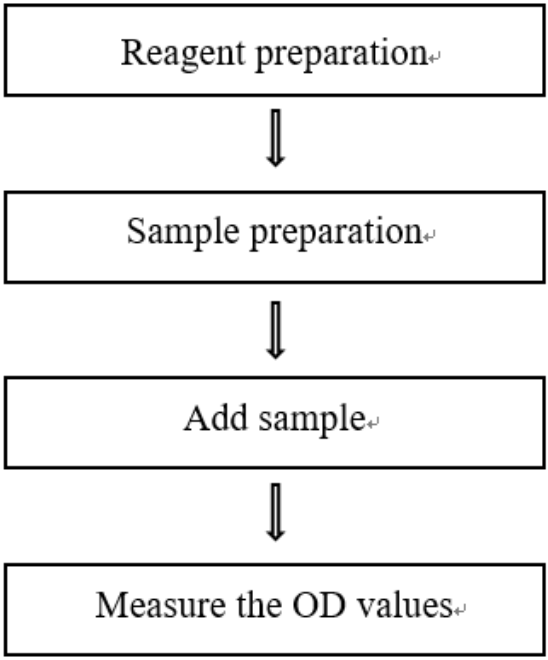
Assay summary



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 this enzyme was calculated by measuring the production rate of cis-aconitase.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	50 mL ×1 vial	50 mL ×2 vials	-20℃, 12 months
Reagent 2	Extraction Solution B	25 mL ×1 vial	50 mL ×1 vial	-20℃, 12 months
Reagent 3	Protease Inhibitor	0.7 mL ×1 vial	1.4 mL ×1 vial	-20 ºC, 12 months, shading light
Reagent 4	Buffer Solution	15 mL ×1 vial	30 mL ×1 vial	-20℃, 12 months
Reagent 5	Substrate	0.15 mL ×2 vials	0.15 mL ×4 vials	-20 ºC, 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

Reagent preparation

Equilibrate all reagents to room temperature before use.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 0.1 g tissue in 900 μL of extraction solution A and 10 μL of protease inhibitor with a dounce homogenizer at 4℃.

④ Centrifuge the homogenized tissue at 600×g for 5 min at 4 ℃, then take the supernatant and discard the precipitate.

⑤ Then centrifuge the supernatant at 15000×g for 10 min at 4 ℃ and transfer the supernatant to another centrifuge tube.

⑥ The supernatant was used to determine the activity of ACO in cytoplasm. Meanwhile, determine the cytoplasmic protein concentration of supernatant.

⑦ Take the precipitate, add 0.2 mL of extraction solution A and 0.002 mL of protease inhibitor, mix the reagent fully and ultrasonic for 3 min.

⑧ Centrifuge at 15000×g for 10 minutes and take the supernatant and discard the precipitate.

⑨ The supernatant was used to determine the activity of ACO in mitochondria. Meanwhile, determine the mitochondria protein concentration of supernatant.

② Dilution of sample

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 extraction solution B. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① When adding the buffer solution, it should be added slowly by touching the wall to avoid bubbles.

② Control the number of samples within 10 per experiment.

③ Try to use fresh samples for testing, which are easy to be inactivated. Put the processed samples on the ice box as far as possible, and it is recommended to use them up within 2 hours.

④ Substrate is prone to be oxidized and should be covered promptly after each use.

Operating steps

① Incubation of the sample: add the supernatant of sample and substrate at volume ratio of 80: 1(the addition volume of substrate is recommended to be more than 10 μL). Mix fully, and incubate at 37 ℃ for 10 min, and preserve sample on ice for detection.

② Sample well: add 20 μL of sample and 180 μL of buffer solution 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℃ for 4 min, measure the OD value of each well at 240 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.

Calculation

The sample:

Tissue sample:

Unit definition: the enzyme amount of 1 nmol of aconitase generated by 1 mg protein at room temperature is defined as 1 unit.

$$\text{ACO activity (U/mgprot)} = \Delta A \div (3.6 \times 0.6) \times 0.0002 \div T \div 0.02 \div C_{pr} \times f \times 10\text{^}6$$

[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.

T: The time of reaction, 4 min.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L)

10^6： 1 mmol=1×10^6 nmol.

0.02: The volume of sample, mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	7.50	22.60	48.50
%CV	10.0	9.4	9.1

Inter-assay Precision

Three mouse liver tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	7.50	22.60	48.50
%CV	5.3	5.6	6.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	11.2	28.5	53
Observed Conc. (U/L)	11.6	27.9	56.7
Recovery rate (%)	104	98	107

Sensitivity

The analytical sensitivity of the assay is 2.32 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

Example analysis：

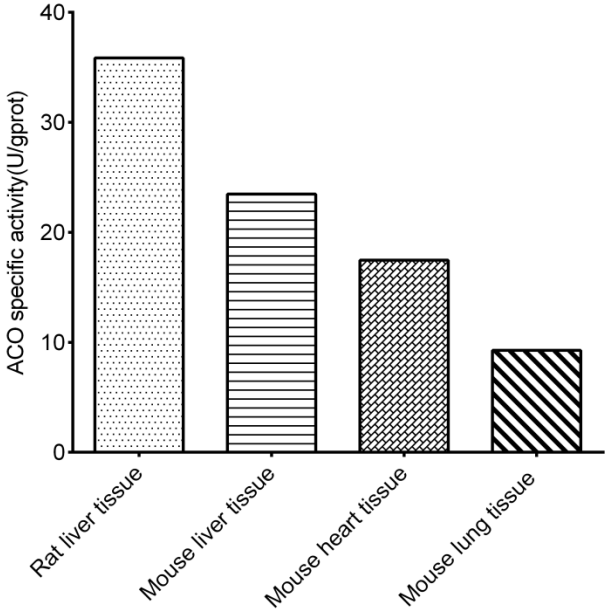
For rat liver tissue, dilute the mitochondria sample of 5% rat liver tissue homogenate for 4 times with extraction solution B, take 20 uL of the diluted sample, and carry the assay according to the operation steps. The results are as follows:

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:

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

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

Detect rat liver tissue homogenate (5% mitochondria protein concentration of supernatant is 9.04 mgprot/L, dilute for 4 times), mouse liver tissue homogenate (5% mitochondria protein concentration of supernatant is 9.07 mgprot/L, dilute for 4 times), mouse heart tissue homogenate (5% mitochondria protein concentration of supernatant is 4.24 mgprot/L, dilute for 4 times) and mouse lung tissue homogenate (5% mitochondria protein concentration of supernatant is 4.31 mgprot/L, dilute for 4 times) according to the protocol, the result is as follows：


40
30
20
10
0
ACO specific activity(U/gprot)
Rat liver tissue
Mouse liver tissue
Mouse heart tissue
Mouse lung tissue

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.