TECHNICAL MANUAL

Aconitase (ACO) Activity Assay Kit

- **SKU CODE:** MAES0263
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Incubation of sample
- Add sample and buffer solution to microplate
- Measure optical density at 240 nm

2. Intended use

This kit can be used to measure aconitase (ACO) activity in animal tissue samples.

3. 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 cis-aconitate in the cell, which plays an important role by maintaining the tricarboxylic acid cycle and glyoxylate cycle.

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

4. 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°C, 12 months
Reagent 2	Extraction Solution B	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months
Reagent 3	Protease Inhibitor	0.7 mL × 1 vial	1.4 mL × 1 vial	-20°C, 12 months, protected from light
Reagent 4	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months
Reagent 5	Substrate	0.15 mL × 2 vials	0.15 mL × 4 vials	-20°C, 12 months, protected from light
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. 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)

6. Reagent preparation

Equilibrate all reagents to room temperature before use.

Note: The reagents must be stored strictly according to the preservation conditions in the table above. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient amounts are obtained.

7. 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°C. ④ Centrifuge the homogenized tissue at 600×g for 5 min at 4°C, then take the supernatant and discard the precipitate. ⑤ Then centrifuge the supernatant at 15000×g for 10 min at 4°C 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): 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 perform a pre-test to confirm the dilution factor.

8. 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 ice as far as possible, and it is recommended to use them within 2 hours.
4. Substrate is prone to oxidation and should be covered promptly after each use.

9. 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°C 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 A1. Incubate at 25°C for 4 min, measure the OD value of each well at 240 nm with microplate reader, recorded as A2. ΔA = A2 - A1.

10. Calculation

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.

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.

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.

11. Appendix I Performance Characteristics

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).

Intra-assay Precision

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.

Inter-assay Precision

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 in parallel to get the average recovery rate of 103%.

Recovery

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

12. Appendix II Example Analysis

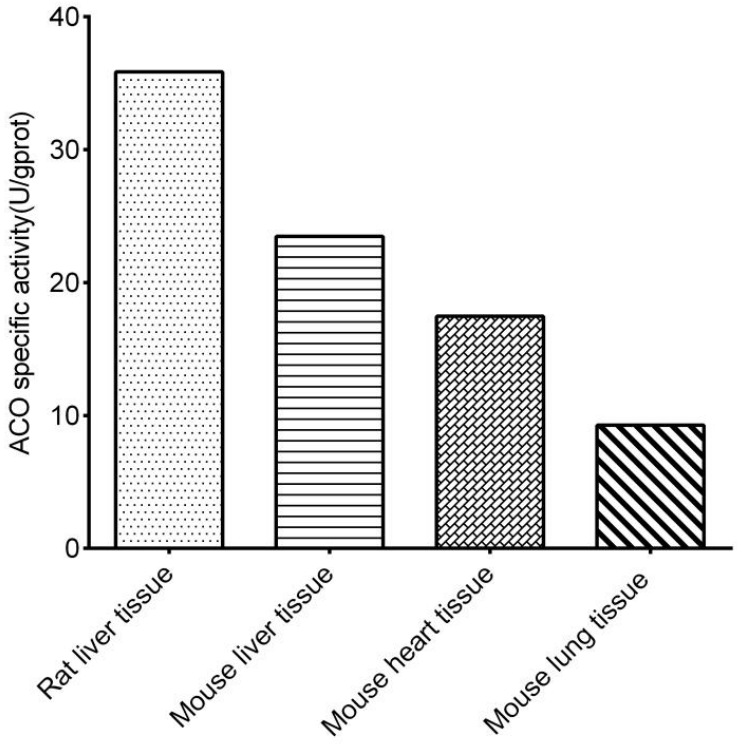
Example analysis:

For rat liver tissue, dilute the mitochondria sample of 5% rat liver tissue homogenate 4 times with extraction solution B, take 20 μL of the diluted sample, and carry out 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:

ACO activity (U/mgprot) = $(0.07 \times 0.0002 \times 4) \div (3.6 \times 0.6 \times 0.02 \times 9.04 \times 4) \times 10^6 = 35.85$ U/mgprot

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



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.