

**112907 Cis-Aconitase Activity Assay Kit - Spectrophotometric Method****Product Introduction**

Aconitase catalyzes the conversion of citrate to isocitrate in the tricarboxylic acid cycle. Citrate is not readily oxidized. Through dehydration and hydration reactions, aconitase shifts the hydroxyl group from the  $\beta$ -carbon atom to the  $\alpha$ -carbon atom to form isocitrate, which is readily dehydrogenated and oxidized for the subsequent oxidative decarboxylation reaction.

ACO catalyzes the conversion of citrate to isocitrate, and the oxidative decarboxylation of isocitrate reduces  $\text{NAD}^+$  to generate NADH, increasing light absorption at 340 nm.

Sample detection results may vary depending on the detection conditions and instrument. Any figure data are for reference only.

**Package Contents**

Pack Size	Code	Component	Quantity
50T	112907.1	Reagent I	1 bottle
50T	112907.2	Reagent II	1 bottle
50T	112907.3	Reagent III	1 tube
50T	112907.4	Reagent IV	1 bottle
50T	112907.5	Reagent V	1 bottle
50T	112907.6	Reagent VI	1 tube
50T	112907.7	Reagent VII	1 tube
50T	112907.m	Instruction Manual	1 copy

**Quality Standards and Safety**

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Reagent I	—	—
Reagent II	—	—
Reagent III	—	—
Reagent IV	—	—
Reagent V	—	—
Reagent VI	—	—
Reagent VII	—	—

**Transportation and Storage**

**Transportation:** Transport with ice packs.

**Storage:** Store Reagent IV, Reagent V, and Reagent VII at 2-8 °C. Store the remaining components at -20 °C. Shelf life: 180 days.

**Instructions for Use****1. Sample Processing**

1. Weigh about 0.1 g tissue or collect 5 million cells. Add 1 mL Reagent I and 10  $\mu\text{L}$  Reagent III, then homogenize using an ice-bath homogenizer or mortar.
2. Transfer the homogenate to a centrifuge tube and centrifuge at 600 g, 4 °C for 5 min.
3. Discard the pellet. Transfer the supernatant to another centrifuge tube and centrifuge at 11000 g, 4 °C for 10 min.

- The supernatant is the cytoplasmic extract and can be used to determine cytoplasmic cis-aconitase activity.
- Add 200  $\mu\text{L}$  Reagent II and 2  $\mu\text{L}$  Reagent III to the pellet from step 4. Disrupt by sonication in an ice bath at 20% power or 200 W, sonicate for 3 s at 10 s intervals, and repeat 30 times. Use this for determination of mitochondrial cis-aconitase activity.

## 2. Reagent Preparation

- Add 3 mL distilled water to Reagent VI and dissolve thoroughly before use. Prepare fresh and use immediately.
- Add 36 mL Reagent IV to Reagent VII and dissolve thoroughly before use.
- Working solution: before use, add 36 mL of Reagent VII, then add 3 mL distilled water, 3 mL Reagent IV, 3 mL Reagent V, and Reagent VI. Mix thoroughly. Prepare fresh and use immediately, or aliquot the working solution and store at  $-20\text{ }^{\circ}\text{C}$  for up to one week.

## 3. Assay Procedure

- Preheat the spectrophotometer for more than 30 min and set the wavelength to 340 nm. Use distilled water to zero the instrument.
- Place the working solution in a  $37\text{ }^{\circ}\text{C}$  water bath for mammals or a  $25\text{ }^{\circ}\text{C}$  water bath for other species for 10 min.
- In a 1 mL quartz cuvette, add 100  $\mu\text{L}$  sample and 900  $\mu\text{L}$  working solution. Mix well. Immediately record the absorbance at 340 nm at 20 s as A1 and at 3 min 20 s as A2. Calculate  $\Delta A = A2 - A1$ .

## Activity Calculation

### 1. Based on Protein Concentration

Unit definition: one unit is the amount of enzyme that generates 1 nmol of NADH per minute per mg tissue protein.

$$\text{ACO activity (nmol/min/mg prot)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (V_{\text{sample}} \times \text{Cpr}) \div T = 536 \times \Delta A \div \text{Cpr}$$

### 2. Based on Fresh Weight

Unit definition: one unit is the amount of enzyme that generates 1 nmol of NADH per minute per g tissue.

$$\text{ACO (nmol/min/g fresh weight)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 108 \times \Delta A \div W$$

### 3. Based on Bacterial or Cell Density

Unit definition: one unit is the amount of enzyme that generates 1 nmol of NADH per minute for every  $1 \times 10^4$  bacteria or cells.

$$\text{ACO activity (nmol/min}/10^4\text{cells)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (500 \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 0.722 \times \Delta A$$

Parameter	Definition	Value
$V_{\text{total reaction}}$	Total reaction volume	0.001 L
$\epsilon$	NADH molar extinction coefficient	$6.22 \times 10^3 \text{ L/mol} \cdot \text{cm}^{-1}$
$d$	Cuvette optical path length	1 cm
$V_{\text{sample}}$	Volume of sample added	0.1 mL
$V_{\text{total sample}}$	Total volume of extraction solution added	0.202 mL
$T$	Reaction time	3 min
$\text{Cpr}$	Sample protein concentration	mg/mL
$W$	Sample mass	g
500	Total number of bacteria or cells	500 ten thousand

## Precautions

- This 50T kit can test 48 samples.
- Required instruments and supplies: UV spectrophotometer, water bath, benchtop centrifuge, adjustable pipette, 1 mL quartz cuvette, mortar, ice, and distilled water.
- Before formal measurement, perform a preliminary test using 2-3 samples with relatively large expected differences.