Cis-Aconitase Activity Assay Kit

Product Code: 112910

112910 Cis-Aconitase Activity Assay Kit - Microplate Method

Product Introduction

Aconitase catalyzes the conversion of citrate to isocitrate in the tricarboxylic acid cycle. Because citrate is not easily oxidized, aconitase enables dehydration and hydration reactions that transfer the hydroxyl group from the β-carbon atom to the α-carbon atom, forming isocitrate. This product is then readily dehydrogenated and oxidized, preparing for the subsequent oxidative decarboxylation reaction.

ACO catalyzes the conversion of citrate to isocitrate, and oxidative decarboxylation of isocitrate reduces $NAD^+$to produce NADH, resulting in increased light absorption at 340 nm.

Representative results obtained with this kit may vary under different assay conditions and with different instruments. Any figure data are for reference only.

Packing List

Pack Size	Code	Item	Quantity
100T	112910.1	Reagent One	1 bottle
100T	112910.2	Reagent Two	1 bottle
100T	112910.3	Reagent Three	1 vial
100T	112910.4	Reagent Four	1 bottle
100T	112910.5	Reagent Five	1 bottle
100T	112910.6	Reagent Six	1 vial
100T	112910.7	Reagent Seven	1 vial
100T	112910.m	Instructions	1 copy

Quality Standards and Safety Information

Raw Materials and Packaging Name	Quality Standard	Main Toxicity
Reagent One	—	—
Reagent Two	—	—
Reagent Three	—	—
Reagent Four	—	—
Reagent Five	—	—
Reagent Six	—	—
Reagent Seven	—	—

Transportation and Storage

Transportation: This product is transported with ice packs.

Storage: Reagents 4, 5, and 7 should be stored at 2-8°C. The remaining components should be stored at -20°C. Shelf life: 180 days.

Instructions for Use

1. Sample Processing

1. Weigh approximately 0.1 g of tissue or collect 5 million cells. Add 1 mL of Reagent 1 and 10 uL of Reagent 3, 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 precipitate, transfer the supernatant to another centrifuge tube, and centrifuge at 11000 g, 4°C for 10 min.
4. The resulting supernatant is the cytoplasmic extract and can be used to determine cytoplasmic cis-aconitase activity.
5. Add 200 uL of Reagent 2 and 2 uL of Reagent 3 to the precipitate from step 1.4, then disrupt by ultrasonication in an ice bath (power 20% or 200 W; sonicate for 3 s, interval 10 s, repeat 30 times) for determination of mitochondrial cis-aconitase activity.

2. Reagent Preparation

1. Add 1.5 mL distilled water to Reagent VI and fully dissolve before use. Prepare fresh before use.
2. Add 12 mL Reagent IV to Reagent VII and fully dissolve before use.
3. Prepare the working solution by mixing 12 mL Reagent VII, 1 mL distilled water, 1 mL Reagent IV, 1 mL Reagent V, and 1 mL Reagent VI thoroughly. Prepare fresh before use, or aliquot and store at -20°C for up to one week.

3. Assay Procedure

1. Preheat the microplate reader for more than 30 min and set the wavelength to 340 nm.
2. Place the working solution in a 37°C (mammals) or 25°C (other species) water bath for 10 min.
3. In a 96-well plate, add 40 μL sample and 160 μL working solution. Mix well and immediately record the absorbance at 340 nm at 20 s as A_1, and the absorbance at 3 min 20 s as A_2. Calculate $\Delta A = A_2 - A_1$.

4. Cis-Aconitase Activity Calculation

4.1 Calculated by Protein Concentration

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

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

4.2 Calculated by Sample Fresh Weight

Unit definition: One enzyme activity unit is defined as the amount that generates 1 nmol of NADH per minute per g of tissue.

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

4.3 Calculated Based on Bacterial or Cell Density

Unit definition: One enzyme activity unit is defined as the amount that generates 1 nmol of NADH per minute per 10^4 bacteria or cells in the reaction system.

ACO activity (nmol/min/10^4cells) = $[\Delta A \times V_{total\ reaction} \div (\varepsilon \times d) \times 10^9] \div (500 \times V_{sample} \div V_{total\ sample}) \div T = 0.216 \times \Delta A$

Parameter	Value
$V_{total\ reaction}$	Total volume of the reaction system, 2×10^{-4}L
ε	NADH molar extinction coefficient, 6.22×10^3L/mol·cm^{-1}
d	96-well plate optical path, 0.5 cm
V_{sample}	Added sample volume, 0.04 mL
$V_{total\ sample}$	Added extraction solution volume, 0.202 mL
T	Reaction time, 3 min
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g
500	Total number of bacteria or cells, 500 × 10,000

Precautions

1. This 100T kit can assay 96 samples.
2. Required instruments and supplies: microplate reader, water bath, benchtop centrifuge, adjustable pipettes, 96-well plate, mortar, ice, and distilled water.
3. Before the formal assay, be sure to select 2-3 samples with expected large differences for a preliminary test.