# Aconitase (ACO) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/microplate reader

**Cat No:** NA0275

**Size:**100T/96S

**Components:**

Reagent I: Liquid 120 mL×1. Storage at 4℃.

Reagent II: Liquid 50 mL×1. Storage at 4℃.

Reagent III: Liquid 0.6 mL×3. Storage at -20℃ and protect from light. Volatile reagent, tighten the lid as soon as possible after use and store at -20℃.

Reagent IV: Liquid 25 mL×1. Storage at 4℃.

Reagent V: Powder×1. Storage at 4℃.

Reagent VI: Powder×1. Storage at 4℃.

**Product Description:**

Aconitase (ACO) is an important intracellular ferritin, which is mainly present in the cytoplasm and mitochondria. ACO catalyzes the reversible reaction of intracellular citric acid to isocitric acid via cis aconitic acid, which plays an important role in maintaining the success of the tricarboxylic acid cycle and the glyoxylic acid cycle.

Aconitase can catalyze isocitrate to produce aconitic acid. Cisaconitic acid has a characteristic absorption peak at 240 nm. The enzyme activity is calculated by measuring the rate of cisaconitic acid production.

**Reagents and Equipments Required but Not Provided:**

Spectrophotometer/microplate reader, water bath, desk centrifuge, water bath, adjustable transferpettor, ultrasonic cell pulverizer, micro quartz cuvette/96 well(UV) plate, mortar/homogenizer, ice and distilled water.

**Procedure:**

**I. Complex extraction:**

A. Extraction of total aconitase:

Collect 0.1 g of tissue or 5 million bacteria( cells) add 1 mL of Reagent I and 10 μL of Reagent III, grinding on ice with mortar/homogenizer and ultrasonic crushing of bacteria or cells (ice bath, 40% power , ultrasonic of 3s, 9 s of interval, repeat for 15 times). Centrifuge at 11000 ×g and 4℃ for 15 minutes, take the supernatant and place it on ice for testtotal aconitase (If calculated in terms of protein concentration, leave a sufficient amount to determine the protein concentration, shorthand as Cpr1. It is recommended to test the sample after diluting 4-10 times with distilled water).

B. Extraction of cytoplasm and mitochondrial aconitase:

Collecting 0.3 g of tissue or 15 million cells, add 1.5 mL of Reagent I and 15 μL of Reagent III, grinding on ice with mortar/homogenizer. After centrifuge at 600 ×g for 5 minutes at 4℃ (If calculated in terms of protein concentration, leave a sufficient amount to determine the protein concentration, shorthand as Cpr2). Take the supernatant to other tube and centrifuge at 11000 ×g for 15 minutes at 4℃ to seprate supernatant and sediment again.The supernatant can used to detect ACO that in mitochondria (It is recommended to test the sample after diluting 4-10 times with distilled water). Add 600 μL of Reagent II and 6 μL of Reagent III to the sediment, splitting with ultrasonication (power 40%, work time 3s, interval 9s, repeat 15 times). Centrifuge at 5000 ×g and 4℃ for 2 minutes, take the supernatant and place it on ice for test,which is used to detect the enzyme activity of ACO in cytoplasm (It is recommended to test the sample after diluting 4-10 times with distilled water).

Note: Select the total aconitase, cytosolic aconitase or mitochondrial aconitase according to the experimental needs.

**II. Detection**

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 240 nm, set zero with distilled water.

2. Sample detection:

(1) Working solution : Before use, pour Reagent V and Reagent VI to Reagent IV, dissolves completely for test. The unused reagent store at 4℃ in the dark.

(2) Preheat the working solution in 25℃ water bath for 15 minutes.

(3) Add the following reagents in turn to micro quartz cuvette / 96 well plate(96 UV plate) .

| Reagent Name (μL) | Test tube |
|---|---|
| Working solution | 180 |
| Sample | 20 |
| Timing after add sample, mix thoroughly. Detect the absorbance at 240 nm at the time of 10 seconds record as A1. Then place dishes with the reaction solution in a 25℃water bath or incubator for 5 minutes. Take the micro quartz cuvette / 96 well plate(96 UV plate) out and wipe it clean, immediately measure the absorbance at the time of 310 seconds which record as A2. calculate ΔA = A1 - A2. | |

**III. Calculation:**

**A. micro quartz cuvette**

a. Calculation of total aconitase

(1) Calculation according to protein content

Definition of unit: One unit is defined as an enzyme activity that per milligram of tissue protein catalyze the generates 1 nmol of maleic acid per minute.

AAO activity (U/mg prot) = $[\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^6] \times (V_S \div Cpr) \div T \times D = 555.55 \times \Delta A \div Cpr \times D$

(2) Calculation by fresh weight of sample

Definition of unit: One unit is defined as an enzym e activity that per gram of tissue catalyze the generates 1 nmol of maleic acid per minute.

AAO activity (U/g fresh weight)= $[\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^6] \times (V_S \div V_E \div W) \div T \times D = 561.11 \times \Delta A \div W \times D$

(3) Bacteria or cultured cells:

Unit definition: One unit of enzyme activity is the amount of 1 0000 cells or bacteria generates 1 nmol of maleic acid per minute.

AAO activity(U/$10^4$ cell)= [ΔA×$V_{RV}$÷(ε×d)×$10^6$]×($V_S$ ÷$V_E$÷N) ÷T ×D = 561.11×ΔA÷N×D

ε: Maleic acid molar extinction coefficient, 3.6 L/mmol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,1×$10^{-3}$ L;

Vs: Sample volume, 0.02 mL;

$V_E$: Extract volume, 0.404 mL;

Cpr1: Sample protein concentration (mg/mL);

T: Reaction time , 5 minutes;

W: Sample weight(g);

N: The number of cells or bacteria, ;

D: Dilution factor;

$10^6$: 1 mmol =$10^6$ nmol.

b. Calculation of mitochondrial aconitase

(1) Calculation according to protein content

Definition of unit: One unit is defined as an enzyme activity that per milligram of tissue protein catalyze the generates 1 nmol of - per minute.

AAO activity (U/mg prot) = [ΔA×$V_{RV}$÷(ε×d)×$10^6$]×($V_S$ ÷Cpr) ÷T ×D= 555.55×ΔA÷Cpr2×D

(2) Calculation by fresh weight of sample

Definition of unit: One unit is defined as an enzym e activity that per gram of tissue catalyze the generates 1 nmol of - per minute.

AAO activity (U/g fresh weight)= [ΔA×$V_{RV}$÷(ε×d)×$10^6$]×($V_S$ ÷$V_E$÷W) ÷T ×D= 561.11×ΔA÷W××D

(3) Bacteria or cultured cells:

Unit definition: One unit of enzyme activity is the amount of 1 0000 cells or bacteria generates 1 nmol of - per minute.

AAO activity(U/$10^4$ cell)= [ΔA×$V_{RV}$÷(ε×d)×$10^6$]×($V_S$ ÷$V_E$÷N) ÷T ×D = 561.11×ΔA÷÷N×D

ε: Maleic acid molar extinction coefficient, 3.6 L/mmol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,1×$10^{-3}$ L;

Vs: Sample volume, 0.02 mL;

$V_E$: Extract volume, 0.404 mL;

Cpr2: Sample protein concentration (mg/mL);

T: Reaction time , 5 minutes;

W: Sample weight(g);

N: The number of cells or bacteria, ;

D: Dilution factor;

$10^6$: 1 mmol =$10^6$ nmol.

c. Calculation of cytoplasm aconitase

(1) Calculation according to protein content

Definition of unit: One unit is defined as an enzyme activity that per milligram of tissue protein catalyze the generates 1 nmol of - per minute.

AAO activity (U/mg prot) = $[\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^6] \times (V_S \div Cpr) \div T \times D = 555.55 \times \Delta A \div Cpr2 \times D$

(2) Calculation by fresh weight of sample

Definition of unit: One unit is defined as an enzym e activity that per gram of tissue catalyze the generates 1 nmol of - per minute.

AAO activity (U/g fresh weight)= $[\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^6] \times (V_S \div V_E \div W) \div T \times D = 244.44 \times \Delta A \div W \times D$

(3) Bacteria or cultured cells:

Unit definition: One unit of enzyme activity is the amount of 1 0000 cells or bacteria generates 1 nmol of - per minute.

AAO activity(U/104 cell)= $[\Delta A \times V_{RV} \div (\varepsilon \times d) \times 10^6] \times (V_S \div V_E \div N) \div T \times D = 244.44 \times \Delta A \div N \times D$

ε: Maleic acid molar extinction coefficient, 3.6 L/mmol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,$1 \times 10^{-3}$ L;

Vs: Sample volume, 0.02 mL;

$V_E$: Extract volume, 0.404 mL;

Cpr2: Sample protein concentration (mg/mL);

T: Reaction time , 5 minutes;

W: Sample weight(g);

N: The number of cells or bacteria, ;

D: Dilution factor;

$10^6$: 1 mmol =$10^6$ nmol.

**B. 96 UV well plate**

Change the d-1 cm in the above formula to d-0.6 cm (the cuvette light path) for calculation.

**Note:**

1. If A>1, please dilute the sample to appropriate concentration, multiply dilute times in the formular.
2. If A<0.01, prolong the enzymatic reaction time and pay attention to calculation formula changes.
3. As the extract solution contains protein (about 1 mg/mL), when measuring protein concentration all of this protein needs to be deducted during measurement.

**Experimental Examples:**

1. Take 0.1g of eragrostis sample, add 1mL Reagent One and 10μL Reagent Three to extract total cis-aconitase, take the supernatant and dilute 4 times, The calculation by the micro quartz cuvette is: ΔA=A2-A1=0.5693-0.5571=0.0122， calculate the enzyme activity according to sample weight:

ACO Activity（U/g weight）=$561.11 \times \Delta A \div W \times N$=273.8217 U/g weight。

2.Take 0.1g of Rabbit kidney sample, add 1mL Reagent One and 10μL Reagent Three to extract total cis-aconitase, take the supernatant and dilute 8 times, The calculation by the micro quartz cuvette is: ΔA=A2-A1=0.4520-0.4186=0.0334， calculate the enzyme activity according to sample weight:

ACO Activity（U/g weight）=561.11×ΔA÷W×N=1499.2859 U/g weight。

**Related Products:**

NA0812/NA0570 α-Ketoglutarate Dehydrogenase(α-KGDH) Activity Assay Kit

NA0799/NA0558 Succinate Dehydrogenase(SDH) Activity Assay Kit

NA0837/NA0595 Pyruvate Dehydrogenase(PDH) Activity Assay Kit

NA0716/NA0475 Isocitrate Dehydrogenase Mitochondrial(ICDHm) Activity Assay Kit