

## **Aconitase Microplate Assay Kit**

**Cat #: orb707327 (manual)**

Detection and Quantification of Aconitase (Aco) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

*For research use only. Not for diagnostic or therapeutic procedures.*

## INTRODUCTION

Aconitase (EC 4.2.1.3), or Aco, is an enzyme in the citric acid (TCA) cycle that catalyzes the conversion of citrate to isocitrate. The activity of aconitase depends largely upon the iron-sulfur  $[\text{Fe}_4\text{S}_4]^{2+}$  cluster. Related diseases include aconitase deficiency (e.g. myopathy and exercise intolerance), Friedreich's ataxia and diabetes.

The assay measures the isocitrate generated as a product of the aconitase reaction. The isocitrate is then oxidized producing NADPH and the oxidation product. The enzyme catalysated reaction product NADPH can be measured at a colorimetric readout at 340 nm.

**KIT COMPONENTS**

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	18 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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**Note:**

**Enzyme:** add 1 ml distilled water to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

## SAMPLE PREPARATION

### 1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 16000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

### 2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 16000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

### 3. For other biological fluids samples

Add 0.1 ml fluids samples into 0.9 ml Assay Buffer, shock. Centrifuged at 11000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

### 4. For mitochondria

Add 1 ml Assay Buffer to the mitochondria precipitation, shock. Centrifuged at 11000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

**ASSAY PROCEDURE**

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 $\mu$ l	--
Distilled water	--	--	200 $\mu$ l
Substrate	180 $\mu$ l	--	--
Enzyme	10 $\mu$ l	--	--
Sample	10 $\mu$ l	--	--

Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.

**Note:**

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

## CALCULATION

**Unit Definition:** one unit of aconitase activity is defined as the enzyme produce 1 nmol NADPH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{Aco (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) \\ & / T \\ &= 4000 \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Aco (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W / \\ & V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{Aco (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times N / \\ & V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of sample

$$\begin{aligned} \text{Aco (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 4000 \times (\text{OD}_{\text{Sample (130S)}} - \text{OD}_{\text{Sample (10S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L} = 400 \text{ nmol/ml}$ ;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l} = 0.2 \text{ ml}$ ;

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

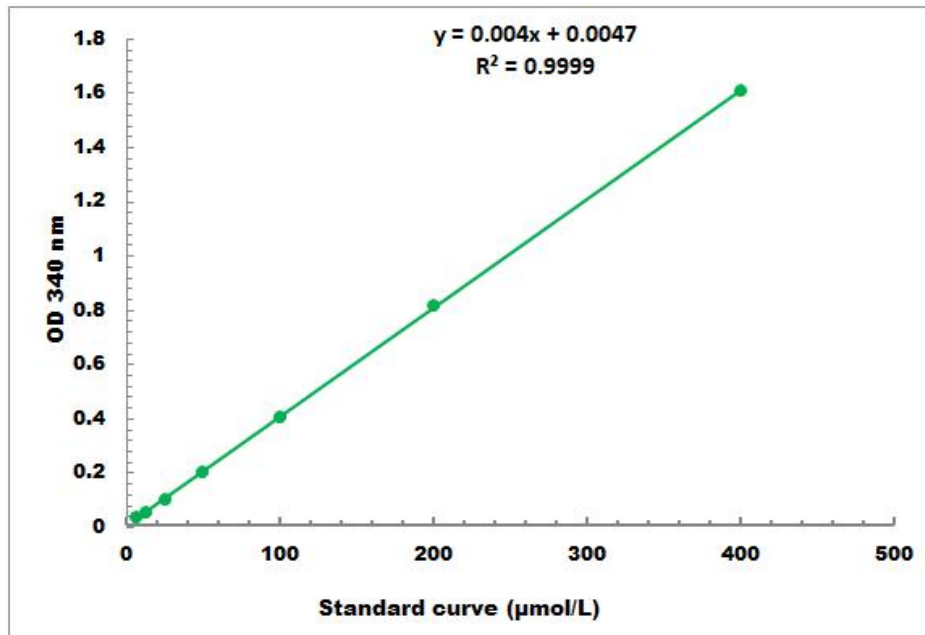
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

## TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L