Aconitase Activity Assay Kit

Catalog No.: abx092298

Size: 96 tests

Detection Range: 2.32 U/L – 60.19 U/L

Sensitivity: 2.32 U/L

Storage: Store all components at -20°C. Store the Protease Inhibitor and Substrate in the dark.

Application: For the detection and quantification of aconitase (ACO) activity in animal tissue homogenates.

Introduction

Aconitase is an important enzyme in the tricarboxylic acid cycle, which is found mainly in the cytoplasm and mitochondria. Aconitase catalyses the conversion of citrate to isocitrate, via the intermediate product cis-aconitate. Cis-aconitate has an absorbance peak at 240nm, and the change in absorbance can be used to calculate the activity of aconitase.

Kit components

1. 96-well UV microplate
2. Buffer solution: 30 ml
3. Substrate solution: 4 x 0.15 ml
4. Extraction Solution A: 2 x 50 ml
5. Extraction Solution B: 50 ml
6. Protease Inhibitor: 1.4 ml
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (240 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Weigh approximately 0.1 g of tissue and wash with pre-chilled PBS (0.01 M, pH 7.4). For each 0.1 g of tissue, add 900 µl of Extraction Solution A and 10 µl of Protease Inhibitor. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 600 x g at 4°C for 5 minutes. Collect the supernatant and discard the pellet. Centrifuge the supernatant again at 15000 x g at 4°C for 10 minutes, then transfer the supernatant to a fresh tube. This supernatant can be used to determine the ACO activity in the cytoplasmic fraction, while the precipitate can be used to determine the ACO activity in the mitochondrial fraction. To prepare the mitochondrial fraction, add 0.2 ml of Extraction Solution A and 0.002 ml of protease inhibitor, mix thoroughly and ultrasonicate for 3 minutes. Centrifuge at 15000 x g for 10 at 4°C for 10 minutes, then discard the precipitate. The protein content of the supernatant should be determined separately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution B, then carry out the assay. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat Liver Tissue Homogenate	6 - 10
10% Rat Lung Tissue Homogenate	6 - 10
10% Mouse Kidney Tissue Homogenate	4 - 6
10% Mouse Liver Tissue Homogenate	6 - 10
10% Mouse Muscle Tissue Homogenate	2 - 4
10% Mouse Brain Tissue Homogenate	4 - 6

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturation that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

Equilibrate all reagents to room temperature before use.

Notes:

- When adding buffer solution, it should be added slowly by touching the walls of the vessel with the pipette tip to avoid bubbles.
- It is recommended to use no more than 10 samples per experiment.
- Fresh samples should be used for each experiment, and should be kept on ice throughout. Use within 2 hours of preparation.
- The substrate is prone to oxidation and should be used as quickly as possible.

B. Assay Procedure

1. Mix the supernatant of the sample and the Substrate at a ratio of 80:1 (eg. add 10 µl of Substrate to 790 µl of sample). Mix thoroughly, incubate at 37°C for 10 minutes, and then store on ice.
2. Add 20 µl of sample and 180 µl of buffer solution to each sample well. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
3. Mix fully with a microplate shaker for 3 seconds.
4. Measure the OD value of each well at 240 nm with a microplate reader. This value should be recorded as A_1.
5. Incubate at 25°C for 4 minutes and measure the OD value of each well at 240 nm with a microplate reader. This value should be recorded as A_2.

C. Calculation of Results

One unit of enzyme activity is defined as the amount of enzyme in 1 mg of total protein that is needed to generate 1 nmol of cis-aconitate in 1 minute at room temperature.

$$\text{ACO activity (U/mg prot)} = \frac{\Delta A \times 0.0002 \times f \times 1{,}000{,}000}{3.6 \times 0.6 \times T \times 0.02 \times C_{protein}}$$

where:

$\Delta A = \Delta A_2 - \Delta A_1$

3.6 = The molar absorption coefficient (L/mmol/cm)

0.6 = Optical path (cm)

0.0002 = Total volume of reaction system (L)

T = Time of reaction (4 min)

f = dilution factor of sample before test

$C_{protein}$ = Concentration of protein in sample

f = The dilution factor of sample

D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.