

Aconitase Assay Kit (Colorimetric)

LS-K199-100 (100 Tests) • Store at -20°C



Introduction

ACONITASE (ACONITATE HYDRATASE) 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 [Fe4S4]2+ cluster. Related diseases include aconitase deficiency (e.g. myopathy and exercise intolerance), Friedreich's ataxia and diabetes.

LSBio's aconitase assay measures the isocitrate generated as a product of the aconitase reaction. The isocitrate is then oxidized producing NADPH and the oxidation product. The NADPH converts the dye to an intense violet color with an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to aconitase activity.

Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 0.5 to 100 U/L for 20 min reaction.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Aconitase activity determination in biological samples (e.g. cell lysate, tissue homogenate, serum, etc.)

Components

Component	K199-100
	100 Tests
Assay Buffer	10 mL
NADP/MTT	1 mL
Standard	1 mL
Enzyme A	120 µL
Enzyme B	120 µL
Substrate	1 mL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipette), clear flat bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Aconitase Assay Kit (Colorimetric)

LS-K199-100 (100 Tests) • Store at -20°C



Assay Procedure

Sample Preparation

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 μ L cold PBS. Centrifuge at 800 x g for 10 min at 4°C. Remove supernatant for mitochondrial preparation.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold PBS. Centrifuge at 800 x g for 10 min at 4°C. Remove supernatant for mitochondrial preparation.

Mitochondrial Preparation: Centrifuge the removed supernatant at 20,000 x g for 10 min at 4°C. Remove the supernatant and resuspend the pellet in cold PBS and sonicate for 20 seconds. The sample can be stored at -80°C for at least one month.

Reagent Preparation

Keep thawed Enzyme A and B on ice and equilibrate all other reagents to 25°C. Briefly centrifuge tubes before use.

Procedure using 96-well plate

1. Standards. Prepare 200 μ L 5000 μ M Premix by mixing 10 μ L of the Standard (100 mM) and 190 μ L distilled water. Dilute standards in 1.5- mL centrifuge tubes as described in the Table. Transfer 20 μ L Standards into separate wells of a clear flat bottom 96-well plate.

No	Premix + H ₂ O	Isocitrate (μ M)
1	100 μ L + 0 μ L	5000
2	60 μ L + 40 μ L	3000
3	30 μ L + 70 μ L	1500
4	0 μ L + 100 μ L	0

2. Transfer 20 μ L of each sample into separate wells. If any samples have high dehydrogenase activity or isocitrate levels, a sample blank will be needed. Transfer 20 μ L of the sample into another well.
3. Prepare enough Working Reagent (WR) for sample and standard wells by mixing, for each well: 8 μ L NADP/MTT Solution, 1 μ L Enzyme A, 1 μ L Enzyme B, 5 μ L Substrate and 70 μ L Assay Buffer. Fresh reconstitution of the WR is recommended. If Sample Blanks are needed, prepare enough Blank Working Reagent (BWR) for the sample blank wells by mixing, for each well: 8 μ L NADP/MTT Solution, 1 μ L Enzyme B, and 75 μ L Assay Buffer.
4. Add 80 μ L WR or BWR to the corresponding wells. Tap plate briefly to mix.
5. Incubate at room temperature. Use a plate reader to read OD_{565nm} at 10 minutes and 30 minutes.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Aconitase Assay Kit (Colorimetric)

LS-K199-100 (100 Tests) • Store at -20°C

Calculations

Subtract blank value (water, #4) from the standard values at 10 min and plot the DOD against standard concentrations. Determine the slope and calculate the aconitase activity of the sample as follows

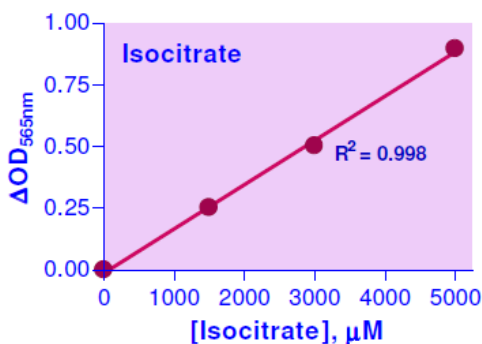
$$\text{Aconitase Activity} = \frac{\text{OD}_{30} - \text{OD}_{10}}{t \text{ (min)} \cdot \text{Slope} (\mu\text{M}^{-1})} \times n \quad (\text{U/L})$$

where OD₃₀, OD₁₀ are optical density values of the Sample at 30 and 10 minutes, respectively. *t* is the reaction time (20 minutes), Slope is the slope of the isocitrate standard curve and *n* is the sample dilution factor.

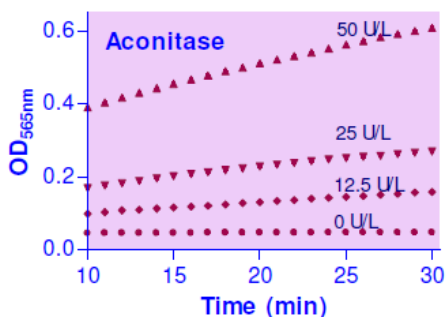
Unit Definition: 1 Unit (IU) of aconitase will catalyze the conversion of 1 μmole of citrate to isocitrate per minute at pH 7.4.

Note: if the calculated activity is higher than 100 U/L, dilute sample in water and repeat assay. Multiply the result by the dilution factor.

Sample Data



Isocitrate Standard Curve



Aconitase Kinetics Graph

Version: V.08.09.2018

FOR RESEARCH USE ONLY! Not for use in humans.