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Product Information

Aconitase Assay Kit

Catalog Number **MAK337**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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 $[Fe_4S_4]^{2+}$ cluster. Related diseases include aconitase deficiency (e.g. myopathy and exercise intolerance), Friedreich's ataxia, and diabetes.

The Aconitase Assay Kit measures the isocitrate generated as a product of the aconitase reaction which includes NADP and MTT. The isocitrate is oxidized, producing NADPH and the oxidation product. The NADPH converts the MTT dye to an intense violet color formazan with an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to aconitase activity.

This kit is suitable for aconitase activity determination in biological samples (e.g., cell lysate, tissue homogenate, serum, etc.).

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Assay Buffer Catalog Number MAK337A	10 mL
NADP/MTT Solution Catalog Number MAK337B	1 mL
Enzyme A Catalog Number MAK337C	120 µL
Enzyme B Catalog Number MAK337D	120 µL
Substrate Catalog Number MAK337E	1 mL
Standard Catalog Number MAK337F	1 mL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 96 well flat bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader
- Reagents and equipment for Sample Preparation

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store components at –20 °C upon receiving.

Preparation Instructions

Reagent Preparation

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

Sample Preparation

Tissue – Prior to dissection, rinse tissue in phosphate buffered saline, pH 7.4, to remove blood. Homogenize tissue (50 mg) in ~200 µL of cold PBS. Centrifuge at 800 × *g* for 10 minutes at 4 °C. Remove supernatant for mitochondrial preparation.

Cell Lysate – Collect cells by centrifugation at 2,000 × *g* for 5 minutes at 4 °C. For adherent cells, use a cell scraper or rubber policemean to harvest cells; do not use proteolytic enzymes. Homogenize or sonicate cells in an appropriate volume of cold PBS. Centrifuge at 800 × *g* for 10 minutes at 4 °C. Remove supernatant for mitochondrial preparation.

Mitochondrial Preparation – Centrifuge the removed supernatant at 20,000 × *g* for 10 minutes 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.

Procedure

Isocitrate Standards

Prepare 200 μL of 5,000 μM Premix by mixing 10 μL of the Standard (100 mM) and 190 μL of ultrapure water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table 1.

Table 1.
Preparation of Isocitrate Standards

Tube	5,000 μM Premix	Ultrapure Water	Isocitrate (μM)
1	100 μL	0 μL	5,000
2	60 μL	40 μL	3,000
3	30 μL	70 μL	1,500
4	0 μL	100 μL	0

Reaction Mix

For each Sample and Standard well, prepare Reaction Mix by mixing:

8 μL NADP/MTT Solution
1 μL Enzyme A
1 μL Enzyme B
5 μL Substrate
70 μL Assay Buffer

Preparation just prior to use is recommended.

Blank Reaction Mix

If Sample Blanks are needed, prepare enough Blank Reaction Mix for the sample blank wells by mixing for each well:

8 μL NADP/MTT Solution
1 μL Enzyme B
75 μL Assay Buffer

Assay Reaction

1. Transfer 20 μL of standards into separate wells of a clear, flat bottom 96 well plate.
2. Transfer 20 μL of each sample into separate wells. Note: If any samples have high dehydrogenase activity or isocitrate levels, a sample blank will be needed. Transfer an additional 20 μL of the sample into a second well.
3. Add 80 μL of Reaction Mix or Blank Reaction Mix (if required) to the corresponding wells. Tap plate to mix briefly and thoroughly. Use of a multichannel pipettor is recommended.
4. Set a timer and incubate plate at room temperature.
5. Measure the absorbance at 565 nm (A_{565}) after 10 and 30 minutes.

Results

Subtract the blank value (Standard 4) from the standard values at 10 minutes and plot the A_{565} against standard concentrations. Determine the slope and calculate the aconitase concentration of Sample as follows:

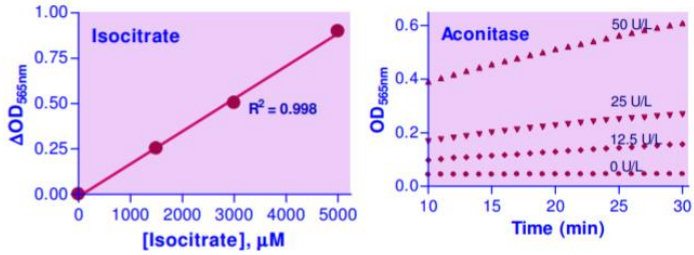
$$\text{Aconitase (U/L)} = \frac{(A_{565})_{30} - (A_{565})_{10}}{T\ (\text{min}) \times \text{Slope}\ (\mu M^{-1})} \times n$$

$(A_{565})_{30}$ = the optical density of the sample at 30 minutes
$(A_{565})_{10}$ = the optical density of the sample at 10 minutes
T (min) = the reaction time (20 minutes)
n = 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 ultrapure water and repeat assay. Multiply the result by the dilution factor.

Figure 1.



Isocitrate Standard Curve *Aconitase Kinetics Graph*

References

1. Kennedy, M.C. et al. The Role of Iron in the Activation/Inactivation of Aconitase. J. Biol. Chem., **258(18)**, 11098-105 (1963).
2. Kennedy, M.C. et al., Purification and characterization of cytosolic aconitase from beef liver and its relationship to the iron responsive element binding protein. Proc. Natl. Acad. Sci. USA., **89(24)**, 11730-34 (1992).
3. Villafranca, J.J. et al., The Mechanism of Aconitase Action. J. Biol. Chem., **249(19)**, 6149-55 (1974).

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