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Product Information

Aconitase from porcine heart

Product Number **A 5384**
Storage Temperature -0 °C

Product Description

Enzyme Commission (EC) Number: 4.2.1.3
CAS Number: 9024-25-3
Molecular weight: approximately 66 kDa (gel filtration)¹
pI: 8.1-8.5²

This enzyme catalyses the stereo-specific isomerization of citrate to isocitrate via cis-aconitate in the tricarboxylic acid cycle.³ This enzyme is found in all cells, since it is required for this early step of the citric acid cycle. Most aconitase is found in the mitochondria, the site for the citric acid cycle. It is inhibited by its product, isocitrate, and activated by its substrate, citrate. This enzyme contains an internal Fe-S cluster required for activity. Therefore, iron is required as a cofactor. This enzyme is strongly inhibited by cyanide and sulfide.⁴ It is also inhibited by copper and mercury ions at low concentrations as well as competitively inhibited by trans-aconitate. At enzyme equilibrium, citrate, aconitate, and isocitrate are present in the proportions 89.5:4.3:6.2. This is obtained irrespective of pH when between pH 6.8 and the optimal pH of the enzyme, pH 7.4. Magnesium ions shift the equilibrium towards citrate.

This product is prepared by ethanol fractionation, carboxymethylcellulose ion-exchange chromatography, ammonium sulfate fractionation, and isoelectric focusing. This procedure involves stabilizing the enzyme during preparation by tricarballylate.⁵ Another method of preparation for this enzyme is also described.⁴

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

This product is soluble in 0.1 M Tris buffer, pH 7.4 (7.5 mg/ml). The following method is used to prepare activated aconitase. Dissolve 15 mg of enzyme in 2.0 ml of 0.1 M Tris buffer, pH 7.4. Add 50 µl of 1 mM ferrous ammonium sulfate (0.39 mg/ml in water) and 0.1 ml of 0.05 M L-cysteine (7.90 mg/ml in water, adjusted to pH 7.4 with NaOH). Incubate for 1 hr at 0 °C. The aconitase is now activated and ready for use.

Storage/Stability

This product is relatively unstable, particularly in solution.⁴ It can be partially stabilized by the addition of citrate. It will lose activity if dialyzed to remove salt.

References

1. Kennedy, S. C., et al., On pig heart aconitase. *Biochem. Biophys. Res. Commun.*, **47(4)**, 740-745 (1972).
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3. Beinert, H., and Kennedy, M.C., Engineering of protein bound iron-sulfur clusters. A tool for the study of protein and cluster chemistry and mechanism of iron-sulfur enzymes. *Eur. J. Biochem.*, **186**, 5-15 (1989).
4. Anfinsen, C.B., Aconitase from Pig Heart Muscle. *Methods in Enzymology*, **1(115)**, 695-698 (1955).
5. *Biochem. Biophys. Res. Comm.*, **47(4)**, 740-745 (1972).

CMH/NSB 1/03

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