Citrate synthase (EC 2.3.3.1), *Escherichia coli*

Catalogue number:

AE00041, 2500 U (148 mg)

Description

Citrate synthase (E.C. 2.3.3.1) is purified from a recombinant *E. coli* strain. The enzyme exists in nearly all living cells and stands as a pace-making enzyme in the first step of the Krebs Cycle. Citrate synthase is localized within eukaryotic cells in the mitochondrial matrix, but is encoded by nuclear DNA rather than mitochondrial. It is synthesized using cytoplasmic ribosomes, then transported into the mitochondrial matrix. Citrate synthase is commonly used as a quantitative enzyme marker for the presence of intact mitochondria. Citrate synthase catalyses the condensation reaction of acetyl-CoA and oxaloacetate producing citrate. Oxaloacetate will be regenerated after the completion of one round of the Krebs Cycle. Oxaloacetate is the first substrate to bind to the enzyme. This induces the enzyme to change its conformation, and creates a binding site for the acetyl-CoA. Only when this citroyl-CoA has formed will another conformational change cause thioester hydrolysis and release coenzyme A. This ensures that the energy released from the thioester bond cleavage will drive the condensation. The enzyme is provided in 3.2 M ammonium sulphate. Swirl the enzyme mix immediately prior to use.

Purity

Citrate synthase has been determined to be >95% pure, according to SDS polyacrylamide gel electrophoresis (PAGE) followed by Coomassie Blue staining (Figure 1).

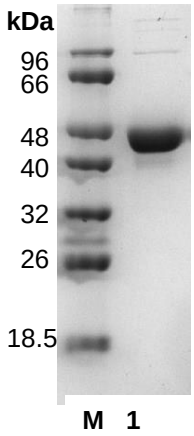

Figure 1. SDS-PAGE analysis of *E. coli* citrate synthase. Electrophoresis was performed using a 12% polyacrylamide gel. Lane M, molecular weight marker; Lane 1, purified citrate synthase (48 kDa).

Storage temperature

Citrate synthase should be stored at 2 °C to 8 °C.

Temperature and pH optimum

The optimum pH and temperature are 8.0 and 25 °C, respectively.

Specific activity

16.9 U/mg, 186 U/ml.

Unit definition

One unit is defined as the amount of enzyme required to produce 1 μmol of citric acid from oxaloacetic acid and acetyl-CoA measured at 232 nm, in a reaction mixture containing 95 mM Tris/HCl buffer (pH 8.0), 0.16 mM oxaloacetic acid and 0.2 mM acetyl-CoA.

Substrate specificity

Under the reaction conditions specified the enzyme might present a minor NADH oxidase activity.

V2202

Certificate of Analysis

Test	Criteria	Result
Protein purity	Purity in line with the stated value	Meets specification
Protein concentration	Concentration in line with the stated value	Meets specification
Catalytic activity	Activity in line with the stated value	Meets specification
Blank assay variability	Absorbance values with less than 10% of variability	Meets specification

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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