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Protocol • Biotin-Protein-Ligase BI001 / BI002

Description

Biotin-protein ligase (EC 6.3.4.15) activates biotin to form biotinyl 5' adenylate and transfers the biotin to biotin-accepting proteins. It also functions as a biotin operon repressor. The protein is encoded by the birA gene.

Other names for this enzyme include biotin ligase, biotin operon repressor protein, birA, biotin holoenzyme synthetase and biotin-[acetyl-CoA carboxylase] synthetase.

Catalog No.	Description
BI001	40 µg; 2 vials of 20 µg each (200,000 Units)
BI002	300 µg; 10 vials of 30 µg each (1,500,000 Units)

Source *E. coli*

Storage buffer 50 mM imidazole, pH 6.8, 50 mM NaCl, 5% glycerol, 5 mM mercaptoethanol.

Storage conditions

The enzyme should be immediately stored at –80°C. After thawing for use, store the vial at 4°C if it is to be re-used in the near future. For longer term storage a vial of thawed enzyme can be safely re-frozen by dropping into liquid nitrogen before storing at –80°C.

Biomix-A and -B can be stored at –20°C. Thawing and re-freezing these mixtures does not cause damage.

Stability Biotin ligase retains >90% of its activity for >3 months when stored at 4°C.

Concentration 0.288 mg/ml by A_{280}.

Purity >98% by Coomassie staining.

Activity: 5,000 Units/µg

Definition of Activity 1 Unit is the amount of enzyme that will biotinylate 1 pmol of peptide substrate in 30 minutes at 30°C using the reaction buffers provided and 38 µM peptide substrate*. The peptide substrate used in the enzyme assays was a 15-mer variant of sequence #85 identified by Schatz (1).

Contaminating proteases: <0.01% as chymotrypsin-like activity.

Instructions

Components provided

- Biomix-A (10X concentration: 0.5 M bicine buffer, pH 8.3)
- Biomix-B (10X concentration: 100 mM ATP, 100 mM MgOAc, 500 µM d-biotin)
- BirA enzyme
- Additional d-biotin

The final reaction mixture should contain: 1 part Biomix-A, 1 part Biomix-B and 8 parts substrate solution.

The amount of birA enzyme to add to the reaction mix may need to be varied to achieve biotinylation within a reasonable time-frame (see below). Typically, for every 10 nmol of substrate (at 40 µM), 2.5 µg of birA enzyme is recommended to complete the biotinylation in 30–40 min. at 30°C.

Note

Various reagents commonly present in biological buffers can inhibit the activity of birA enzyme. These include NaCl (100 mM), glycerol (5%) and ammonium sulfate (50 mM). Consequently, the concentration of these reagents in the substrate solution should be minimized. If possible, add the substrate to the reaction mix in 10 mM Tris-HCl, pH 8.

To ensure a rapid rate of biotinylation, it is recommended that the substrate be as concentrated as possible in the final reaction mix (up to 40 µM). The lower the substrate concentration in the reaction mix, the longer it will take to biotinylate. For example, whereas a substrate at 40 µM may be biotinylated in ~30 minutes, at 4 µM it will take ~5 hours using the same amount of birA enzyme. To perform the biotinylation in 30 min. (i.e. 10 times faster), it is necessary to add 10 times more enzyme to the reaction mix.

Biomix-A and -B have been optimized for the biotinylation of substrates at concentrations of no more than 40 µM. If it is desired to biotinylate substrate at concentrations of 40–80 µM, then it is necessary to supplement the reaction mix with additional biotin as follows: 1 part Biomix-A, 1 part Biomix-B, 7 parts substrate solution, 1 part supplemental biotin. For substrate concentrations above 80 µM, please contact GeneCopoeia Technical Service at (301) 762-0888.

The reaction conditions described above have been optimized for a 15-mer peptide similar to sequence #85 identified by Schatz (1). We have investigated the optimum reaction conditions for substrates in which the biotin peptide tag is attached to a protein and found that they are identical to the reaction conditions for the peptide substrate.

Reference

1. Schatz, P.(1993) Biotechnology 11, 1138-1143

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