Biotin-Protein Ligase / BirA Enzyme ● Catalog No. BI001

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.

Components

Item	Composition	Amount
Biotin-Protein Ligase (1mg/ml)	Biotin-Protein Ligase in: 50 mM Imidazole, 50 mM NaCl, 5% (v/v) Glycerol, 5 mM Mercaptoethanol, pH 6.8	40 µl
10×Biotin Ligase Buffer A	0.5 M Bicine buffer, pH 8.3	1 ml
10×Biotin Ligase Buffer B	100 mM ATP, 100 mM MgOAc, 500 µM D-biotin	1 ml
Additional D-biotin	500 µM D-biotin	1 ml

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.
10×Biotin Ligase Buffer A and Buffer B can be stored at -20°C. Thawing and re-freezing these mixtures does not cause damage.

Activity

≥7,500 Units/µg of Biotin-Protein Ligase (BirA Enzyme)

Concentration

1.0 mg/mL by A280 (Extinction coefficient; 1.349 AU at 280nm λ=1 mg/mL Biotin Protein Ligase).

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 40 µM peptide substrate*.
*The peptide substrate used in the enzyme assays was a 27kDa AviTag'd recombinant protein espressed by Pichia pastoris.

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

Recommanded Conditions for Biotin-Protein Ligase (BirA Enzyme)

The final mixture should contain:

Component	Volume	Final Concentration
10×Biotin Ligase Buffer A	2.5 µl	1×
10×Biotin Ligase Buffer B	2.5 µl	1×
Biotin-Protein Ligase	0.17 µl	6.4 ng/µl
Substrate	Variable (1 nmol total)	40 µM
dH_2O	up to 25 µl	

The amount of Biotin-Protein Ligase to add to the reaction mixture may need to be varied to achieve biotinylation within a reasonable time-frame . Typically, for every 1 nmol of substrate (at 40 µM), 1.5µl of Biotin-Protein Ligase is recommended to complete the biotinylation in 30~40 min. at 30°C.

Guidelines for Biotin-Protein Ligase (BirA Enzyme)

1. Various reagents commonly present in biological buffers can inhibit the activity of Biotin Protein Ligase. 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 8.0.

2. 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 to 40 µM may be biotinylated in about 30 minutes, at 4 µM it will take about 5 hours using the same amount of Biotin-Protein Ligase. 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.

3. 10×Biotin Ligase Buffer A and Buffer 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 of Buffer A, 1 part of Buffer B, 7 parts of substrate solution, 1 part of supplemental biotin. For substrate concentrations above 80 µM, please dilute the substrate with 10 mM Tris-HCl, pH 8.0

4. 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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