



Biotin-Protein Ligase - BIRA

Specifications

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.

Source: *E. coli*

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

Storage conditions: The enzyme arrives on dry ice and should be immediately stored at -80°C . After the vial is thawed it should be stored at 4°C if it is to be re-used in the near future. For long-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 . There is no problem in thawing and re-freezing these mixtures.

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

Concentration: 3.0 mg/mL by A_{280} .

Purity: >98% by Coomassie staining.

Activity: >8000 Units/ μg

Definition of Activity: 1 Unit is the amount of enzyme that will biotinylate 1 pmol of peptide substrate* in 30 min. at 30°C using the reaction buffers provided and 38 μM peptide substrate*.

* the peptide substrate use in the enzyme assays was a 15-mer variant of sequence #85 identified by Schatz (1).

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

Reference:

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

Instructions for Use

Components provided:

Biomix-A
Biomix-B
BirA enzyme
Additional 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 of the substrate within a reasonable time-frame (see below). Typically, for every 10 nmol of substrate (at 40 μ M), we recommend 2.5 μ g of birA enzyme to complete the biotinylation in 30-40 minutes at 30°C.

It should be noted that various reagents commonly present in biological buffers can inhibit the activity of birA enzyme. These include NaCl, glycerol and ammonium sulfate. Consequently, the concentration of these reagents in the substrate solution should be minimized. We recommend that if possible, the substrate be added 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 μ M). The lower the substrate concentration in the reaction mix, the longer it will take to biotinylate. For example, whereas a substrate at 40 μ M may be biotinylated in ~30 minutes, it will take ~5 hours at 4 μ M using the same amount of birA enzyme. To bring this reaction time down to 30 minutes at 4 μ M (10 times faster), you would need 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 μ M. If it is desired to biotinylate substrate at concentrations of 40-80 μ M, 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 μ M, please contact our Technical Help at (720) 859-6111.

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.