



Biotin-Protein Ligase (BirA500)

Enzyme 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. Molecular weight of biotin protein ligase is 35,310 daltons.

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 (or, in some cases, ice brix) 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: 1.0 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 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