




Product Data Sheet: Recombinant *E. coli* BirA, Tag Free (Lyophilized)

Product details	
Product Name	: Recombinant <i>E. coli</i> BirA, Tag Free
Product Part Number	: BirA-311
Description	Recombinant BirA, a biotin protein ligase from <i>Escherichia coli</i> (<i>E. coli</i>), is a key enzyme in the biotinylation process. This enzyme specifically catalyzes the transfer of biotin to biotin-accepting proteins and peptides, a crucial step for various biotechnological applications, including affinity purification and detection of biotinylated molecules. BirA biotinylates the lysine side chain of biotin-accepting proteins and peptides, including its natural substrate, carboxyl carrier protein (BCCP), and AviTag fusion proteins.
Synonyms	Biotin-[acetyl-CoA-carboxylase] ligase, <i>E. coli</i> biotin holoenzyme synthetase
Sequence	 <p>*There is a short 11 amino acid overhang post-cleavage. Sequence: Full sequence available upon request. Total Amino Acids : 331 aa. Calculated MW: 35.9 kDa</p>
Formulation	Lyophilized in 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM DTT
Purity & Identity	≥90% pure, Verified by SDS PAGE. Identity verified by Western blotting. Data available upon request.
Features	Specificity: High specificity for biotinylation at lysine residues. Versatility: Suitable for various applications including protein-protein interaction studies, and in vivo biotinylation.
Protein Content	Concentration verified by Total protein assay.
Activity	≥500 Units/μg of BirA.
Definition of Activity	1 Unit (U) of BirA is the amount of enzyme that will biotinylate 1 pmol of Avi Tag substrate at 2.3 μM within 30 minutes at room temperature in a solution containing 10 mM ATP, 10 mM MgOAc, and 50 μM d-biotin.
Application	Biotinylation of target proteins in vitro and in vivo, Protein labeling for affinity purification, Studying protein-protein interactions. Use in various bioanalytical techniques such as ELISA, Western blotting, and immunohistochemistry. Research Use Only. Not for diagnostic or therapeutic applications.



Storage & Handling

Products are lyophilized and shipped at ambient temperature. Please follow the storage and handling instructions below after receiving the product.

Storage	Upon arrival, store the lyophilized protein at -20°C.
Stability	Stable as supplied for 3 months from retest date. For continued use beyond the retest date, contact the manufacturer.
Reconstitution	Gently tap down the vial to ensure that all lyophilisate is collected at the bottom of the vial. Reconstitute the product with the provided 1x BirA buffer. Reconstitute to 0.9 mg/mL with 10% (w/v) glycerol by gently pipetting the solution down the sides of the vial. Avoid vigorous shaking that can cause foaming and protein denaturation. Keep on ice. Aliquot and store at < -70°C for up to 3 months. Avoid repeated freeze-thaw cycles.
Recommendation	As an additional precaution, after adding reconstituted products to media, filter-sterilize before use in cell culture.

Frequently Asked Questions

- What substrates does BirA biotinylate?**
 BirA specifically biotinylates lysine residues within a conserved biotinylation sequence found in its substrate proteins and peptides.
- What are the conditions for optimal BirA activity?**
 Optimal conditions include a reaction buffer containing 10 mM ATP, 10 mM MgOAc, and 50 μM d-biotin at pH within 6.5–8.8. The reaction can happen at room temperature or overnight at 4 °C depending on the stability of your substrate.
- Is BirA cross-reactive with substrates from other species?**
 Yes, BirA can biotinylate substrates from various species as long as they contain the biotin-accepting sequence.
- What is the recommended concentration for BirA in biotinylation reactions?** A BirA concentration of 5 μg/mL is typically sufficient for complete biotinylation of 2.3 μM target proteins and peptides at the aforementioned optimal conditions. Lower concentrations of BirA can be used if a lesser degree of biotinylation is preferred.



References

1. Chapman-Smith, A., & Cronan, J.E. Jr. (2001). The enzymatic biotinylation of proteins: a post-translational modification of exceptional specificity. *Protein Science*, 10(12), 2608-2619.
2. Cronan, J.E. (1989). The *E. coli* biotin regulatory system: transcription of the biotin operon. *Cell*, 58(3), 427-429.
3. Cull, M.G., & Schatz, P.J. (2000). Biotinylation of proteins in vivo and in vitro using small peptide tags. *Methods in Enzymology*, 326, 430-440.
4. Li, Y., & Sousa, R. (2012). Expression and purification of *E. coli* BirA biotin ligase for in vitro biotinylation. *Protein Expression and Purification*, 82(1), 162-167.
5. Fairhead, M., & Howarth, M. (2015). Site-specific biotinylation of purified proteins using BirA. In: Gautier A., & Hinner M.J. (eds) *Site-Specific Protein Labeling*. Methods in Molecular Biology, vol 1266, pp. 171-184.

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End of Product Data sheet