

## BirA Ligase (N-GST)

<b>Catalog Number:</b>	604101, 604102
<b>Size:</b>	25 ug, 100 ug
<b>Target Name:</b>	BirA ligase
<b>Regulatory Status:</b>	RUO

### PRODUCT DETAILS

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<b>Application:</b>	Enzymatic reaction
<b>Format:</b>	Liquid, Purified
<b>Expression Host:</b>	E.coli
<b>Species:</b>	Escherichia coli
<b>Accession Number:</b>	P06709
<b>Sources:</b>	BirA ligase with N-terminus GST is expressed in E.coli cells.
<b>Molecular Weight:</b>	This protein has a predicted molecular weight of 62.2 kDa. Under DTT-reducing conditions, the protein migrates at approximately 65 kDa on SDS-PAGE.
<b>Affinity Tag:</b>	N-GST
<b>Purity:</b>	>95% based on SDS-PAGE under reducing condition
<b>Formulation:</b>	20mM Tris, 300mM NaCL, 5mM DTT, 10% glycerol
<b>Endotoxin level:</b>	Not tested
<b>Protein Concentration:</b>	25µg size is bottled at 0.3mg/mL concentration. 100 µg size is supplied at a lot-specific concentration.
<b>Storage and Handling:</b>	Briefly centrifuge the vial upon receipt. An unopened vial can be stored at 4°C for up to 2 weeks, or at -20°C or below for up to six months. The protein may be further diluted to 0.1 mg/mL using 0.22 µm filtered Tris PH 7.5 buffer. For long-term storage, the diluted stock solution should be aliquoted and stored at ≤ -70°C to minimize freeze-thaw cycles. If additional dilution is required, carrier proteins such as FBS or BSA should be added to maintain protein stability.

### BACKGROUND INFORMATION

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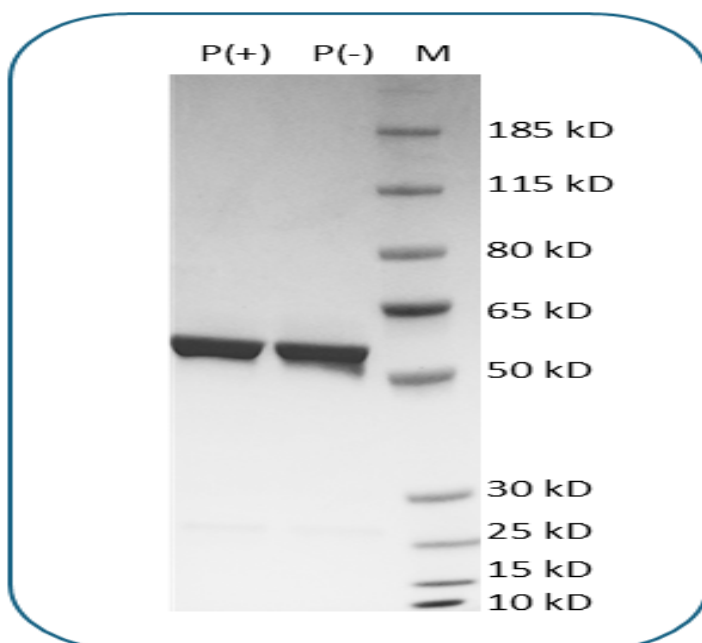
BirA ligase (biotin-protein ligase) is a bacterial enzyme best known for catalyzing the ATP-dependent covalent attachment of biotin to specific lysine residues within target proteins. In its native context in *Escherichia coli*, BirA biotinylates the biotin carboxyl carrier protein (BCCP) subunit of acetyl-CoA carboxylase, a key enzyme in fatty acid synthesis. This post-translational modification is essential for metabolic function, as biotin acts as a cofactor in carboxylation reactions. BirA also functions as a transcriptional repressor in bacteria, regulating genes involved in biotin biosynthesis depending on intracellular biotin levels.

Structurally, BirA is a soluble cytosolic protein of approximately 35 kDa that forms a homodimer under certain functional states. It contains a central catalytic domain responsible for binding biotin and ATP, forming a reactive biotinyl-5'-AMP intermediate. The

enzyme then transfers biotin to the  $\epsilon$ -amino group of a specific lysine within a conserved recognition sequence. Its primary ligands are biotin and ATP, as well as the acceptor protein substrate. Engineered systems commonly use a minimal 15-amino-acid "AviTag" peptide, which BirA recognizes with high specificity.

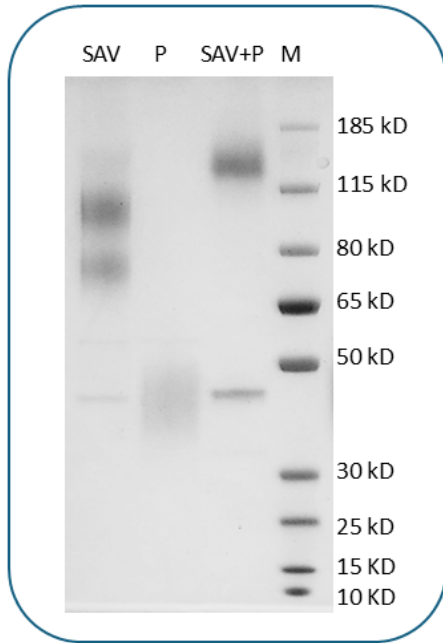
In research and development, BirA is widely used for site-specific protein biotinylation. Recombinant proteins fused to an AviTag can be enzymatically biotinylated either *in vivo* or *in vitro*, enabling uniform, stoichiometric labeling. This precise biotinylation is particularly valuable for applications such as surface plasmon resonance (SPR), ELISA development, flow cytometry reagents, cell-based assays, and structural biology studies. Because the biotin-streptavidin interaction is extremely strong and specific, BirA-mediated labeling provides a robust and versatile tool for immobilization, detection, and multimerization of proteins in both basic research and therapeutic development workflows.

## PRODUCT DATA



Purified BirA (N-GST) final product on SDS-PAGE under non-reducing (P-) and reducing (P+) conditions. The purity of BirA ligase appears to be greater than 95%.

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Human Tim-3 (C-His-Avi) Protein is biotinylated by BirA ligase in vitro in BirA buffer. Based on Gel shift Assay by co-incubation with Streptavidin, biotinylation efficiency is >90% for Biotinylated Tim-3.