



Biotin Protein Ligase

Manual

Catalog #	5031	5033
Package Size	200 µg	1 mg
Concentration	2 µg/µL	2 µg/µL
Volume	100 µL	500 µL

Custom formats and package sizes are available upon request



Important!

-20°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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Table of Contents

Product Description..... 3

Applications..... 3

Benefits..... 3

Source..... 3

Specifications..... 3

Components and Storage Temperature..... 4

Quality Control..... 4

Protocol 4

References..... 5

Related Products 5

Ordering Information..... 5

Technical Support 6

Description:

IG® Biotin Protein Ligase (BirA) is an ATP-dependent enzyme that catalyzes the site-specific covalent attachment of biotin to a specific lysine residue within a compatible biotin-acceptor peptide or protein. The reaction requires biotin, ATP, and magnesium and produces a stable biotinylated protein suitable for downstream detection, immobilization, purification, and interaction studies.

Recombinant *Escherichia coli* BirA is widely used to biotinylate proteins containing short biotin-acceptor sequences, including AviTag™ (GLNDIFEAQKIEWHE), BioTag (ALNDIFEAQKIEWHA), and BirA Substrate Peptide, or BSP (LHHILDAQKMVWNHR). These peptide sequences can be genetically fused to either the N-terminus or C-terminus of a target protein, enabling specific and controlled biotinylation by BirA.

Compared with nonspecific chemical labeling, BirA-mediated biotinylation provides site-specific modification at a defined lysine residue while helping preserve protein structure and biological activity. Biotinylated proteins bind streptavidin, avidin, or neutravidin with high affinity, supporting sensitive and reproducible applications in protein research, assay development, and biopharmaceutical studies.

Applications:

- *In vitro* biotinylation of AviTag-, BioTag-, or BSP-tagged proteins
- Preparation of biotinylated proteins for ELISA and other binding assays
- Protein immobilization on streptavidin-coated surfaces
- Protein–protein and protein–ligand interaction studies
- Preparation of biotinylated antigens, antibodies, receptors, and enzymes
- Affinity purification, pull-down assays, and biosensor applications

Benefits:

- Highly specific enzymatic biotinylation
- Controlled labeling at a defined lysine residue
- Helps preserve protein structure and activity
- Produces stable biotinylated proteins with strong streptavidin binding
- Suitable for a wide range of research and assay-development applications

Source:

Recombinant *E. coli* BirA is fused to the C-terminus of maltose-binding protein (MBP). A His-tag is located at the N-terminus of the fusion protein.

Specifications:

Purified *E. coli* BirA fusion protein with a molecular weight of 78 kDa.

Product Components and Storage Temperature:

The following reagents are supplied with this product:

IG Cat #	Package Size	Component Name	IG Part #	Amount	Concentration	Storage
5031	200 µg	Biotin Protein Ligase	P241302	1 × 100 µL	2 mg/mL	-20 °C
		10x Biotin Ligase Reaction Buffer	P241310	2 × 1.25 mL	10X	-20 °C
		10x Substrate	P241318	2 × 1 mL	10X	-20 °C
5033	1 mg	Biotin Protein Ligase	P241306	1 × 500 µL	2 mg/mL	-20 °C
		10x Biotin Ligase Reaction Buffer	P241314	1 × 12.5 mL	10X	-20 °C
		10x Substrate	P241322	1 × 10 mL	10X	-20 °C

Custom formats and package sizes are available upon request.

Quality Control:

The activity of the supplied Biotin Protein Ligase (BirA) is evaluated using a 20 kDa protein containing an N-terminal AviTag (GLNDIFEAQKIEWHE). Under the recommended reaction conditions, 1 µg of BirA catalyzes greater than 95% biotinylation of 100 µg of the tagged protein in a 50 µL reaction after incubation for 1 hour at 37 °C.

In vitro Biotinylation Protocol:

- Set up the reaction in a 1.5 mL microcentrifuge tube according to the table.

Reagent	µL of Sample
Protein with an AviTag, BioTag, or BSP tag	100 µg
Biotin Protein Ligase	0.5 µL (1 µg)
10x Substrate	10.0 µL
Sterile water	XX µL
Total Volume:	100 µL

Note: The optimal mass ratio of biotin ligase to tagged protein may vary depending on the protein. The ratio described above is a recommended starting point. Test several ratios to determine the conditions that provide the desired level of biotinylation.

- Incubate the mixture at for 1 hour at 37°C.

Note: The reaction may be performed at 30 °C or lower; however, lower temperatures may reduce biotinylation efficiency. Some proteins may require a longer incubation time to achieve the desired level of biotinylation.

- Remove a sample containing 1 µg of protein from the reaction mixture and analyze it by SDS-PAGE. Include a non-biotinylated sample as a negative control. Biotinylation may cause a mobility shift in the tagged protein.

Alternatively, the biotinylation can be assessed by incubating the reaction mixture with excess amount of immobilized streptavidin; biotinylated proteins will be removed by streptavidin and non-biotinylated proteins remain in the solution.

References:

1. Fairhead M, Howarth M. Site-specific biotinylation of purified proteins using BirA. Methods Mol Biol. 2015;1266:171-84. doi: 10.1007/978-1-4939-2272-7_12. PMID: 25560075; PMCID: PMC4304673.

Related Products:

- IG® DsRed Fluorescence Standard Kit (Cat.# 5010)
- IG® mVenus Fluorescence Standard Kit (Cat.# 5014)
- TEV Protease (Cat.# 5022, 5025, 5027)
- BL21(DE3) Chemically Competent Cells (Cat. # 1051-06, 1055-384)
- IG® Autoinduction DE3 Chemically Competent Cells (Cat. # 1065-06, 1065-96)

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
Email: **sales@intactgenomics.com**
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.



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Our hours are Monday - Friday, 8 AM to 5 PM, U.S. central standard time (CST).

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