Product Information

Enzymatic Protein Biotinylation Kit

For biotinylation of up to 20 mg target protein

CS0008

Product Description

Biotinylated proteins are used in various applications, such as binding assays, biopanning, and protein purification. Although chemical biotinylation methods are common, they present several challenges, such as partial labeling and high batch-to-batch variation, because of the random nature of these methods. In addition, the lack of control of the biotinylation site might affect the biotinylated protein's activity.

The Enzymatic Protein Biotinylation Kit allows direct and specific biotinylation using a specific biotin ligation enzyme. The kit contains all the components required for in vitro biotinylation of up to 20 mg recombinant target protein. Biotinylation is mediated by the enzyme BirA biotin ligase, which uniformly ligates biotin to lysine residues in the context of several tags, such as Avi-Tag™ and Biotag™ (see Table 1 for a list of commonly used enzymatic biotinylation tags).

This kit was tested successfully under a wide range of conditions, including:

- Temperatures ranging from 4 °C to 30 °C
- pH from 6.0 to 9.0
- NaCl concentrations from 50 to 500 mM

The kit includes Streptavidin, which can be used to assess the degree of biotinylation. The kit also includes a BLPR-tagged protein as a positive control.

Precaution and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

This kit contains sufficient reagents for the biotinylation of up to 20 mg target protein that contains one of the tags listed in Table 1.

Component	Component Number	Amount	Cap Color
BirA ligase	CS0008A	100 µL	Yellow
ATP	CS0008B	0.5 mL	Red
Biotin	CS0008C	0.5 mL	Green
Magnesium sulfate	CS0008D	0.5 mL	Violet
Control protein	CS0008E	100 µL	Blue
Streptavidin	CS0008F	50 µL	Clear

Component Information

- BirA ligase (CS0008A: Yellow Cap): 1 mg/mL BirA ligase in a 50% glycerol buffered solution. Store at –70 °C, or at –20 °C for up to 6 months. Keep on ice while in use. To avoid multiple freeze/thaw cycles, it is recommended to prepare aliquots upon thawing, and store the aliquots at –20 °C or lower.
- ATP (CS0008B: Red Cap): 200 mM solution. To avoid multiple freeze/thaw cycles, it is recommended to prepare aliquots upon thawing, and store the aliquots at –20 °C or lower.
- Biotin (CS0008C: Green Cap): 10 mM solution in 20 mM Bicine buffer, pH 8.3. Store at –20 °C or lower.
- Magnesium sulfate (CS0008D: Violet Cap): 1 M solution. Upon thawing, this component can be stored at room temperature, –20 °C or at –70 °C.
- Control protein (CS0008E: Blue Cap): Store at –20 °C or lower.

- Streptavidin (CS0008F: Clear Cap): 5 mg/mL buffered solution. Store at -20 °C or lower.

Equipment Required (Not Provided)

- Temperature-controlled incubator, water bath or heat block
- SDS-PAGE equipment and supplies (to test the degree of biotinylation by a gel-shift assay)

Storage and Stability

The kit is shipped on dry ice. Upon receipt, store all components at –70 °C for up to 2 years, or at –20 °C for up to 6 months. The unopened kit is stable for 2 years as supplied.

General Notes

Sequences of Biotin-Receiving Tags

BirA ligase specifically biotinylates certain tags on target proteins. Such tags should be recombinantly added to the protein of interest.

The native E. coli BirA biotin ligase substrate is a 75-amino acid-long peptide that is derived from Biotin Carboxyl Carrier Protein (BCCP).

A few additional peptides were described for BirA-mediated biotinylation and are summarized in Table 1 below.

Table 1. Tags recognized by BirA

Name	Sequence
Consensus[1]	LXZIFEAQKIEWR
Avi-Tag™[2]	GLNDIFEAQKIEWHE
Biotag™[1]	ALNDIFEAQKIEWHA
BLRP[3]	MAGGLNDIFEAQKIEWHEDTGGS
BSP1[1]	LHHILDAQKMVWNHR
BSP2[2]	LCDIFESQKIEWHSAA

X = any amino acid

Z = Any amino acid except L, V, I, W, F, Y

Location of Tag

The tag can be located either at the carboxy or amino terminus of the target protein. The optimal location of the tag depends on the properties of the target protein and should be determined experimentally.

A spacer of several amino acids may be required.

If possible, it is recommended to test several tag configurations (such as amino-terminal or carboxy-terminal, inclusion of a spacer of varying lengths, etc.).

Target Protein Concentration

The rate of the biotinylation reaction is positively dependent on the concentration of the target protein. Therefore, a higher concentration is advisable, if possible.

A protein concentration of at least 1 mg/mL is recommended. The solubility of some proteins may be negatively affected by the biotin conjugation.

If protein precipitation occurs upon biotinylation, reducing the target protein concentration may improve the protein's solubility.

Optimizing the pH and/or ionic strength of the reaction may also improve the solubility of the biotinylated target protein.

Ligation Reaction Conditions

BirA ligase was shown to work under a wide variety of conditions, as described in Table 2.

Table 2. Ligation reaction compatibility

Condition	Range
pH	6.0 - 9.0
NaCl	0 - 500 mM
Temperature	4 - 30 °C
Buffers	MES, Tris, Bicine, PBS
Mg	1 - 10 mM
ATP	5 - 15 mM
Protein concentration	1 - 5 mg/mL

The efficiency of the biotinylation reaction depends on the inherent properties of each target protein, as well as the conformational availability of the tag on the target protein.

Biotin Concentration

It is recommended to use 20-50% molar excess of biotin over the target protein concentration in the reaction mixture. Depending on the molecular weight and concentration of the target protein, this typically translates into 0.1-0.3 mM biotin in the reaction mix.

ATP Concentration

ATP availability is critical for successful biotinylation. It is therefore recommended to use a final ATP concentration of at least 5 mM in the reaction mix.

Procedure

Control Protein Reaction

If desired, the biotinylation reaction can be tested using the supplied control protein (CS0008E, Blue Cap vial):

- Mix all components according to Table 3.
- Incubate for 1 hour at 30 °C.

Table 3. Control protein reaction mix

Reagent	Cap Color	Volume
Control protein	Blue	50 µL
ATP	Red	1.3 µL
Biotin	Green	1.0 µL
Magnesium sulfate	Violet	0.5 µL
BirA ligase	Yellow	1.0 µL

Target Protein Reaction

As each protein has different physicochemical properties, it is suggested to run a set of reactions with different conditions (such as different protein concentrations, pH range, etc.) to obtain optimal biotinylation.

The final suggested target protein concentration range is 1-5 mg/mL, in an appropriate buffer (see Table 2 for reaction compatibility).

As a starting point, use the protocol in Table 4, and the recommended ratios of BirA ligase to the target protein in Table 5.

This protocol can be scaled up or down as needed, depending on the amount of the target protein:

- Please download the Excel sheet calculator, from the CS2000 Product Detail Page.
- Use the Excel sheet to calculate the total accumulating volumes needed for all your reactions (based on Table 4).

Mix all the needed components. Incubate for 1 hour at 30 °C, or for longer at a lower temperature, depending on the stability of the target protein.

Further adjustment of BirA ligase to target protein ratio, incubation time and temperature may be required to achieve optimal biotinylation, according to the experimental results obtained for each target protein.

Use Streptavidin to assess the biotinylation efficiency, as described in the next section.

Table 4. Suggested conditions for biotinylation reaction

Reagent	Cap Color	Final Concentration
Target protein	N/A	1-5 mg/mL
ATP (200 mM)	Red	5 mM
Biotin (10 mM)	Green	0.1 mM
Magnesium sulfate (1 M)	Violet	5 mM
BirA ligase (1 mg/mL)	Yellow	Varies*

Note: See Table 5 for suggested BirA ligase-to-target protein ratios.

Table 5. Recommended ratio of BirA ligase and target protein (mg:mg)

Target Protein Molecular Weight	BirA:Protein Ratio (w/w)	
	1 h at 30 °C	16 h at 4 °C
10-20 kDa	1:50	1:100
20-50 kDa	1:100	1:200
50-100 kDa	1:200	1:400
Above 100 kDa	1:400	1:800

Streptavidin Gel-Shift Assay

To assess the biotinylation efficiency, a gel shift assay with the supplied Streptavidin (CS0008F) may be performed. A fully biotinylated protein will completely shift on SDS-PAGE upon pre-incubation with Streptavidin.

As controls, incubate a sample of non-biotinylated protein, and a sample without Streptavidin and run in parallel. Note that free biotin removal should be performed prior to the analysis (see "Free biotin removal" below).

Gel-Shift Assay Procedure

- Determine the biotinylated protein concentration by appropriate methods (such as Bradford, Lowry, or A280).
- Prepare samples according to Table 6.
- Boil the samples in a dry heat block at 90 °C for 3 minutes. Spin down.
- Cool the samples to room temperature for 3 minutes.
- To the "tested sample" add 2 µL of Streptavidin (Clear cap). Vortex briefly. Spin down.

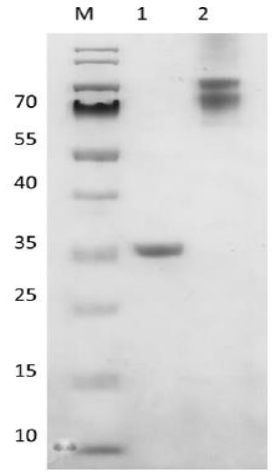
- Incubate for 5 minutes at room temperature. Then analyze both samples on SDS-PAGE.

Table 6. Gel-shift assay sample

Sample	Target Protein	4× Sample Buffer	dH_2O
Target protein	2-10 µL (~10 µg)	10 µL	Complete to 30 µL

Note: Prepare the sample, then split into 2 separate tubes (15 µL per tube). Label one tube as "tested sample" and the other as "no Streptavidin".

Figure 1. Streptavidin gel-shift assay



Lane 1: Control protein, no streptavidin

Lane 2: Control protein with streptavidin

Free Biotin Removal

Binding of streptavidin to the biotinylated protein will be inhibited by excess free biotin. Therefore, free excess biotin should be removed prior to performing any analysis or use of the biotinylated protein.

For large volumes (> 3 mL), dialysis is recommended.

In smaller volumes (≤3 mL), spin buffer exchange columns (such as PAGE-Optimizer™ Sample Preparation Column, Z742615) or micro-dialysis tubes (such as Pur-A-Lyzer™ Mini Dialysis Kit: PURN25005, PURN12100, PURN12050, PURN12030, PURN12010) are recommended.

Note: if the Control protein reaction is performed as described, there is no need to remove excess biotin.

References

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6. Fairhead, M., and Howarth, M., Methods Mol Biol., 1266, 171-184 (2015).

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