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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The *In Vitro* Biotinylation Kit (with BirA Tagless Enzyme) is a kit designed for *in vitro* addition of biotin to biotin-acceptor peptides using BirA. This kit comes in a convenient, easy-to-use format and contains all the components necessary to label biotin-acceptor peptides with biotin. The BirA protein provided is a tagless recombinant biotin protein ligase (EC6.3.4.15). It efficiently catalyzes the specific attachment of biotin to the AviTag™. It is a robust reaction in a wide range of conditions, such as 20-40 µM of protein substrate (biotin-acceptor peptide) concentrations and can tolerate up to 1M NaCl and 20% glycerol in the reaction.

Background

Addition of biotin molecules is commonly used for the labeling and purification of proteins or other target molecules. Biotin labeling takes advantage of the exceptionally strong interaction between biotin (also known as vitamin H) with avidin or streptavidin. The affinity of biotin to avidin or streptavidin is one of the strongest known non-covalent interactions known between a protein and a ligand, exhibiting a dissociation constant (Kd) around $4x10^{-14}$ M. BirA, an *E. coli* biotin protein ligase, covalently adds biotin to an AviTag™ peptide in a highly efficient and specific manner, in a reaction that requires ATP. It has the advantage of resulting in a homogeneous product that can be used in protein capture, immobilization, and functionalization. For more information on enzymatic biotinylation please refer to Enzymatic Biotinylation & BirA (bpsbioscience.com).

Applications

Specific biotin labeling of proteins/peptides containing the Avi-Tag™ sequence for downstream applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100147	Tagless BirA Enzyme (1mg/ml)	2 x 10 µl	-80°C
82513	10x Buffer A	2x 1 ml	4°C -20°C
82514	10x Buffer B	2x 1 ml	4°C -20°C
82515	10x Buffer C	2x 1 ml	4°C -20°C

Materials Required but Not Supplied

- Protein of interest containing an Avi-Tag™
- Water bath/incubator
- All the materials for the detection of the resulting biotinylation level, such as:
  - Streptavidin for gel shift assays
  - Avidin-agarose for avidin pull down assays

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The buffer of the protein of interest should not contain ammonium sulfate, as this component inhibits BirA activity. If present, remove ammonium sulfate by dialysis, or spin columns, to Tris, pH 8.0/ NaCl/ glycerol.

***In Vitro* BirA Biotinylation Protocol**

- We recommend using Spike S1 RBD (V367F), 1.4 mg/ml (#KC100769) as control.

A. Protein Substrate Preparation

1. Concentrate the Avi-Tag™-containing protein or peptide substrate to a concentration ≥ 20 µM and/or perform buffer exchange for a buffer containing Tris, pH 8.0/NaCl/glycerol.
   Note: An Amicon concentrator with appropriate membrane cutoff may be used. Removal of ammonium sulfate can be accomplished by dialysis or spin columns.

B. BirA *In Vitro* Biotinylation Reaction

- If the concentration of protein substrate is > 20 µM but < 40 µM the biotinylation reaction should be composed of 8 parts of protein substrate, 1 part of 10x Buffer A and 1 part of 10x Buffer B.
- If the concentration of protein substrate is > 40 µM the biotinylation reaction should be composed of 7 parts of protein substrate, 1 part of 10x Buffer A, 1 part of 10x Buffer B and 1 part of 10X Buffer C.
- Note that the order of addition can be critical for protein stability. Buffer B is acidic and addition of this buffer before Buffer A can lead to precipitation/denaturing of proteins in solution.

1. Prepare the biotinylation reaction:
   a. If the concentration of protein substrate is > 20 µM but < 40 µM:
      i. Add 8 parts of protein substrate at a concentration of > 20 µM to a tube.
      ii. Add 1 part of 10x Buffer A. The final amount of Buffer A in the reaction will be 1x.
      iii. Add 1 part of 10x Buffer B. The final amount of Buffer A in the reaction will be 1x.

   b. If protein substrate concentration is > 40 µM:
      i. Add 7 parts of protein substrate at a concentration of > 20 µM to a tube.
      ii. Add 1 part of 10x Buffer A. The final amount of Buffer A in the reaction will be 1x.
      iii. Add 1 part of 10x Buffer B. The final amount of Buffer B in the reaction will be 1x.
      iv. Add 1 part of 10x Buffer C. The final amount of Buffer C in the reaction will be 1x.

2. Calculate the amount of protein to use (in nmole) based upon the concentration and the molecular weight of the protein. For aid with calculations please refer to: Lab Calculators (bpsbioscience.com). For example, 100 µg of a 50 kDa protein substrate corresponds to 2 nmoles.

3. Add 1 µg of BirA enzyme for each 2 nmole of protein substrate.

4. Incubate at 30°C for 30 minutes.

   Note: If the protein substrate is sensitive to temperature, an incubation at 4°C overnight with 0.33 µg of BirA per 2 nmoles of protein substrate can be used. If the protein substrate is in a buffer that contains NaCl and/or glycerol, up to 1M NaCl and/or 20% glycerol results in similar biotinylation.

Validation
Streptavidin Gel-Shift

- SDS-PAGE conditions to be used are dependent on the protein of interest and protocols established in the laboratory. The protocol described is a generic protocol only.
- The streptavidin tetramer is a 52 kDa protein, and it runs at ~50-60 kDa as a non-denatured tetramer complex in SDS-PAGE under the conditions necessary to observe a gel shift. The band corresponding to the target protein substrate can easily disappear for proteins smaller than 45 kDa. Larger proteins, > 75kDa, can also be assessed with this method if the gel being used for analysis can readily resolve complexes with streptavidin. If the size of target protein substrate is between 45 to 75 kDa, different percent of SDS-PAGE may help to resolve different mobilities in SDS-PAGE between the original denatured target protein and the folded streptavidin tetramer. Alternatively, an Avidin pulldown can be used for analysis.
- The efficiency of biotinylation can be measured by SDS-PAGE by analyzing the change in intensity of the band of the original protein substrate with and without addition of streptavidin tetramer. When the protein is biotinylated the streptavidin tetramer forms a strong interaction with the biotinylated protein substrate, which is stable during electrophoresis and will result in a larger protein complex, which can be seen by SDS-PAGE.

1. Pipet 40-100 pmole of biotinylated protein substrate into a new tube (for example, a PCR tube).

2. Add 6 µl of 2x SDS-PAGE sample buffer.

3. Heat at 95°C for 5 minutes (for example, in a PCR block with a heated lid).

4. Allow the tube to cool to Room Temperature (RT).

5. Add 2 µl of a 2.6 mg/ml solution of Streptavidin into the sample tube.

6. Incubate the sample at RT for 5 minutes.

7. Run the sample on an appropriate SDS-PAGE gel.

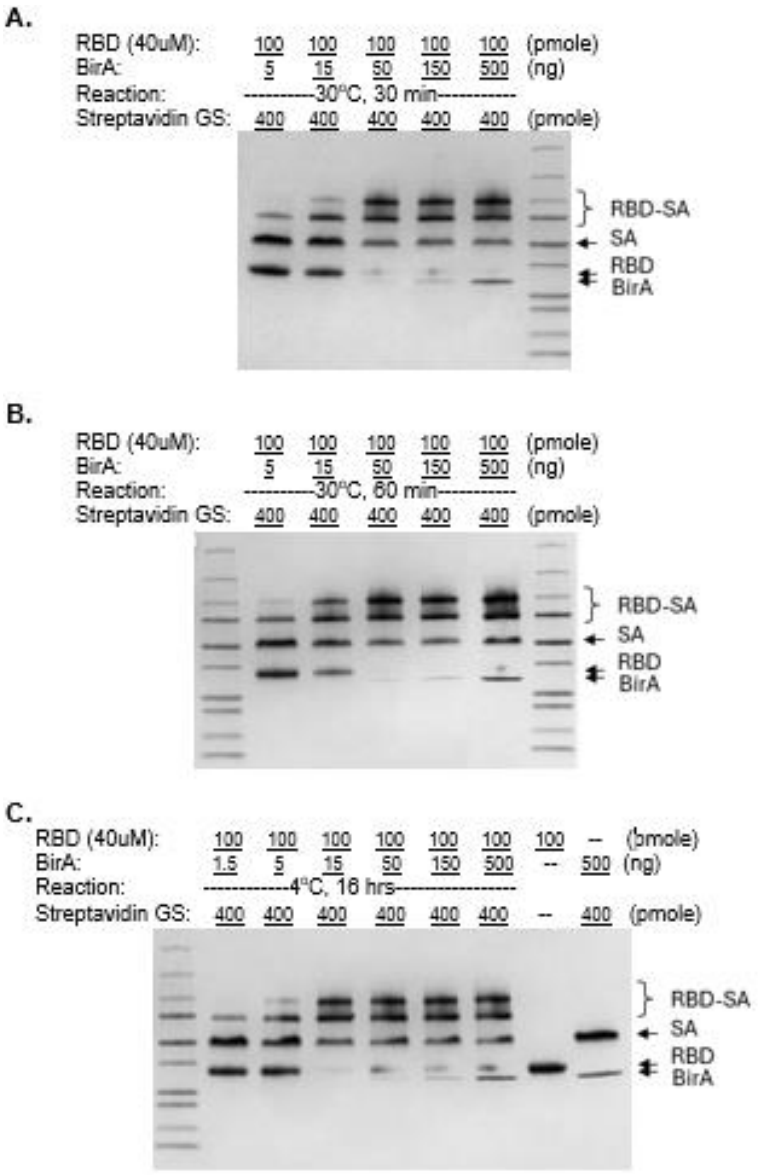

Figure 1. Assessment of the biotinylation efficiency by gel shift when testing BirA titration under different reaction conditions.

A. Biotinylation of RBD-Avi-Tag was performed at 30°C for 30 minutes in the presence of increasing amounts of BirA and measured by gel shift. 50 ng of BirA was sufficient to biotinylate 100 pmole of RBD.
B. Biotinylation of RBD-Avi-Tag was performed at 30°C for 60 minutes in the presence of increasing amounts of BirA and measured by gel shift. <50 ng of BirA was sufficient to biotinylate 100 pmole protein substrate.
C. Biotinylation was performed at 4°C for 16 hours in the presence of increasing amounts of BirA and measured by gel shift. 15 ng of BirA is sufficient to biotinylate 100 pmole of protein substrate.

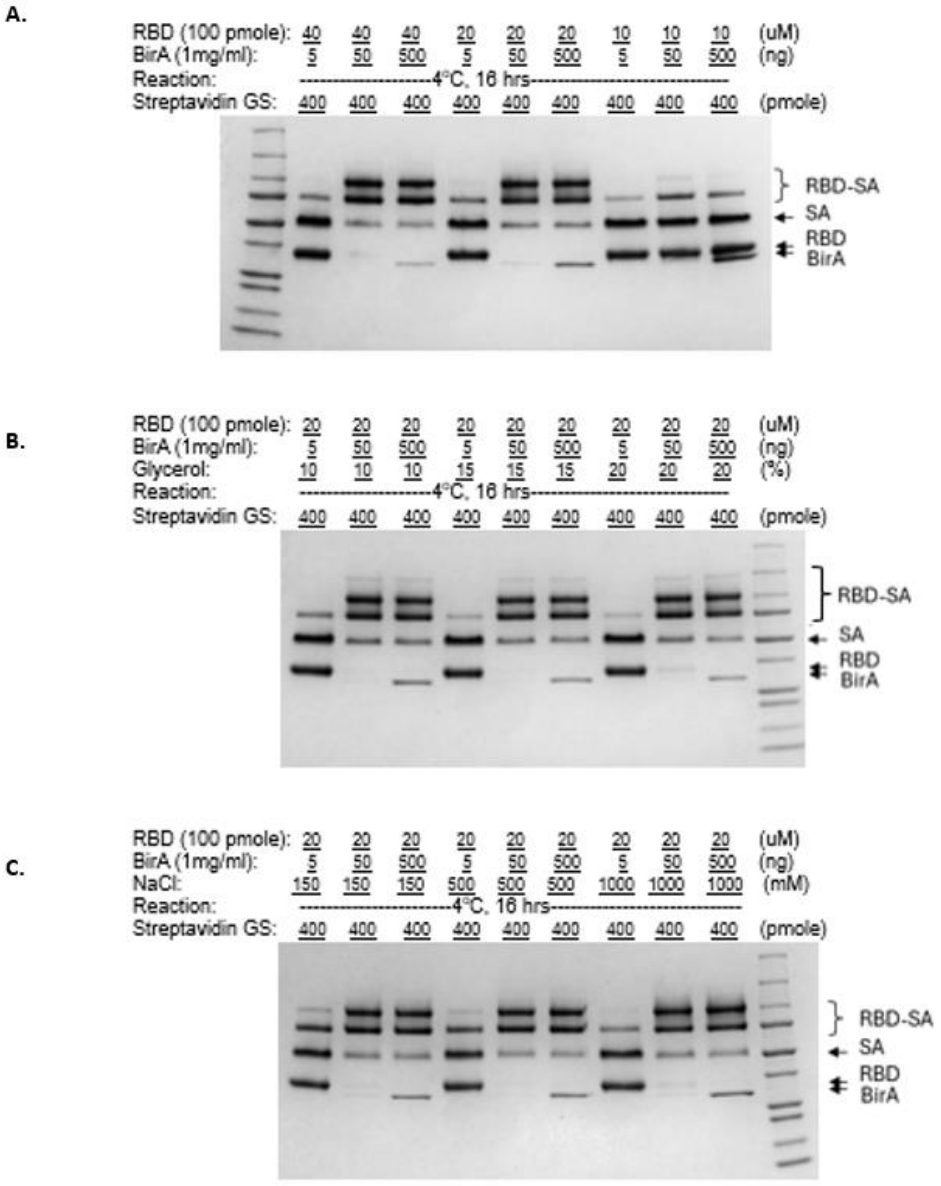

Figure 2. Effect of concentration, glycerol, and NaCl on the biotinylation efficiency of Spike S1 RBD (V367F), Avi-His-tag Recombinant by BirA in vitro analyzed by gel shift.

A. Spike S1 RBD (V367F), Avi-His-tag biotinylation was performed in the presence of 10, 20 and 40 µM of protein substrate and measured by gel shift. Both 20 µM and 40 µM resulted in similar biotinylating efficiency.
B. Spike S1 RBD (V367F), Avi-His-tag biotinylation was performed in the presence of 10%, 15% and 20 % of glycerol and measured by gel shift. All conditions resulted in similar biotinylation efficiency.
C. Spike S1 RBD (V367F), Avi-His-tag biotinylation was performed in the presence of 150mM, 0.5M and 1M of NaCl and measured by gel shift. All conditions resulted in similar biotinylation efficiency.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Spike S1 RBD (V367F), 1.4 mg/ml	KC100769	2x 10 µl
BirA-transformed Competent E. coli cells	27462	10 vials
BirA, GST-Tag (E. coli) (E. coli derived) Recombinant	70031	100 µg
BirA, His-FLAG-tags (E. coli) (E. coli derived) Recombinant	70030	100 µg
BirA *in vivo* Biotinylation Kit (with BirA Competent Cells)	27461	10 x 1L
BirA *in vivo* Biotinylation Kit (without BirA Competent Cells)	78870	10 x 1L

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