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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The *E. coli in vivo* Biotinylation Kit (without BirA Competent Cells) comes in a convenient, easy-to-use format and contains the components necessary to label biotin-acceptor peptides with biotin expressed in *E. coli.* strain BL21, a chemically competent E. coli B strain. This kit contains sufficient reagents to label 10 x 1 L of *E. coli* culture. BirA-Transformed chemically competent *E. coli* cells (BPS Bioscience #27462) are not provided with this kit and must be purchased separately.

Start by transforming the competent cells with a vector expressing your protein of interest linked to an AviTag™ sequence (for example: pAN or pAC vectors). After transformation, BirA expression is induced by IPTG and biotin is added to the culture. BirA efficiently catalyzes the specific attachment of biotin to the AviTag™.

Background

Biotin-labeling is commonly used for non-radioactive labeling and purification of proteins or other target molecules. Biotin labeling takes advantage of the exceptionally strong interaction between biotin (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.

Applications

Specific biotin labeling of proteins/peptides containing the AviTag™ sequence for downstream applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	100x Biotin (5 mM)	10 x 12 ml	-80°C
	1000x IPTG (1M)	10 x 1.3 ml	-80°C
	1000x DTT (1M)	10 x 150 µl	-80°C
	10x Lysis Buffer	10 x 6 ml	Room Temp

Materials Required but Not Supplied

- BirA-Transformed Chemically Competent *E. coli* (BPS Bioscience #27462)
- SOC or LB medium
- Expression vector containing gene of interest and the AviTag™ sequence.
- Streptomycin or spectinomycin, and appropriate antibiotic based on expression vector for the gene of interest.
- Water bath
- Protease inhibitor cocktail (for example, protease cocktail from Sigma #P8465: AEBSF 23 mM, EDTA 100 mM, Bestatin 2 mM, Pepstatin A 0.3 mM, E-64 0.3 mM).
- Affinity chromatography for AviTag™

Stability

This assay kit will perform optimally for up to **12 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.

Protocol

Transformation

It is advantageous to set up a control to determine transformation efficiency by using a control plasmid.

1. Thaw 1 tube of BirA-transformed Chemically Competent *E. coli* cells on ice for each transformation to be performed.

   Note: To increase transformation efficiency, keep cells chilled on ice at all times.

2. Add 1-50 ng of plasmid with the gene of interest into the tube containing the BirA-transformed Chemically Competent *E. coli* cells in a volume no greater than 10 µl per 60 µl of cells.

3. Add 1-10 µl of a control plasmid to another tube of BirA-transformed Chemically Competent *E. coli* cells.

4. Quickly flick the tube several times to ensure even distribution of the DNA.

5. Immediately place tube(s) on ice for 10 minutes.

6. Heat-shock the cells by transferring the tube to a pre-warmed water bath at 42°C for 30 seconds. **Do NOT shake the tube.**

7. Immediately place tubes on ice for 2 minutes.

8. Add 900 µl of pre-warmed (37°C) SOC or LB medium to the cells.

9. Incubate at 37°C with gentle shaking (225 rpm) for 1 hour.

10. Plate 100-200 µl of transformed cells onto plates containing streptomycin or spectinomycin and the appropriate antibiotic for selection of the expression vector.

    Note: The quantity of cells required for plating may vary depending on the transformation efficiency. If you know your plasmid results in a low transformation efficiency, the cells can be pelleted by centrifugation at 1000 x g for 1 min., then resuspended in 50-200 µl of SOC or LB medium and plated.

11. Place plates in 37°C incubator for 14-48 hours.

BirA Induction and Expression

1. Grow 50 ml culture of cells in SOC or LB medium containing either 100 µg/ml of streptomycin or 100 µg/ml of spectinomycin overnight.

2. In the morning, transfer the entire content of the tube into 1 L of culture medium with 100 µg/ml of streptomycin or spectinomycin.

3. Monitor cell growth by measuring OD600.

4. Once OD600 reaches 0.6, add 10 ml of **100x Biotin** plus 1 ml of **1000x IPTG** to the culture to induce BirA expression and catalyze the biotinylation reaction.

5. Allow culture to incubate overnight at 25°C on a shaker.

Cell lysis and protein purification

1. Dilute **10x Lysis Buffer** 10-fold with ice-cold distilled water to make 1x Lysis Buffer.

2. Collect the cell pellet by centrifugation at 6,000 rpm for 10 min, and gently remove the supernatant (culture medium).

3. Wash the cell pellet with 50 ml of PBS and follow with centrifugation at 6,000 rpm for 10 min.

4. Carefully remove the supernatant.

5. Resuspend the cell pellet with >3x volume of 1x Lysis Buffer (usually 30–50 ml per L of culture).

6. Add protease inhibitors and dilute **1000x DTT** 1000-fold in the cell suspension.

7. Lyse cells using a sonicator, cell disruptor or by freeze-&-thaw.

8. Remove cell debris by centrifugation at 10,000 *x g* for 30 min at 4°C.

9. Purify the protein of interest (biotinylated protein) using appropriate affinity chromatography. If the protein contains additional affinity tags (GST, His, FLAG), we recommend purifying first using the affinity tag, and then follow with AviTag™ purification.

Validation Results

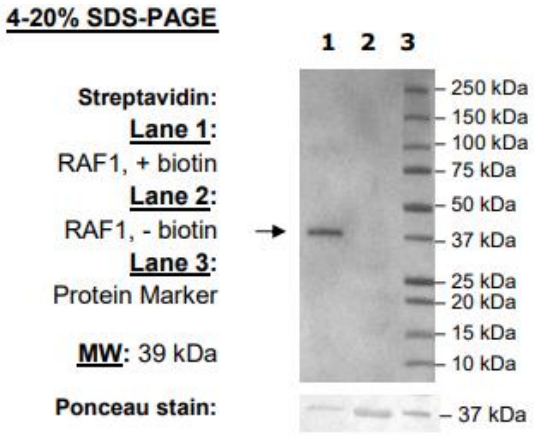

Figure 1. RAF-1-GST-AviTag™ biotinylation using E. coli in vivo Biotinylation Kit.
A RAF1- GST-AviTag™ construct was expressed following the recommended protocol. Lysates were incubated with (Lane 1) or without biotin (Lane 2) and RAF1 was purified using GST-affinity chromatography, followed by avidin-affinity chromatography. Purified RAF1 was tested for biotinylation by SDS-PAGE and Streptavidin-HRP detection.

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*
BirA-transformed Chemically Competent *E. coli* cells	27462	10 vials
BirA, GST-Tag	70031	100 µg
BirA, His-FLAG-Tags (*E. coli* derived)	70030	100 µg
BirA, His-FLAG-Tags (SF9 derived)	70032	50 µg
E. coli in vivo Biotinylation Kit	27461	10 x 1L*