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Data Sheet

***E. coli in vivo* Biotinylation Kit**

Catalog # 27461

Size: This kit contains sufficient reagents
to label 10 x 1 L of *E. coli* culture

BACKGROUND: Biotin-labeling is commonly used for non-radioactive labeling and purification of proteins and other target molecules. Biotin labeling takes advantage of the exceptionally strong interaction between biotin (vitamin H) and either avidin or streptavidin. The affinity of biotin to avidin or streptavidin is one of the strongest known non-covalent interactions of a protein and ligand, exhibiting a dissociation constant (K_d) around 10^{-15} . BirA protein ligase covalently adds biotin to biotin-acceptor peptides containing an AviTag™ sequence in a highly efficient and specific manner in a reaction that requires ATP.

DESCRIPTION: The *E. coli in vivo biotinylation kit* comes in a convenient, easy-to-use format and contains all the components necessary to label biotin-acceptor peptides with biotin in *E. coli*. Strain BL21, a chemically competent *E. coli* B strain. The cells include an IPTG-inducible plasmid containing the BirA gene and the resistance gene for streptomycin or spectinomycin. Just transform the competent cells with a vector expressing your protein of interest with an AviTag™ sequence (example: pAN or pAC vectors). After transformation, BirA expression is induced and biotin is added to the culture. The BirA efficiently catalyzes the specific attachment of biotin to the AviTag. This kit contains sufficient reagents to label 10 x 1 L of *E. coli* culture.

COMPONENTS:

Catalog #	Component	Amount	Storage	
27462	BirA-transformed Chemically Competent <i>E. coli</i>	10 x 50 μ l	-80°C	(Avoid freeze/ thaw cycles!)
	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 μ l	-80°C	
	10x Lysis Buffer	10 x 6ml	room temp	

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

- SOC or LB medium
- Expression vector containing gene of interest and 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, cat. #P8465: AEBSF 23 mM, EDTA 100 mM, Bestatin 2 mM, Pepstatin A 0.3 mM, E-64 0.3 mM)
- Affinity chromatography for AviTag

APPLICATIONS: Useful for specific biotin labeling of proteins/peptides containing the AviTag sequence for downstream applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE: Fairhead, M, and Howarth, M. [Methods Mol Biol. 2015; 1266: 171–184.](#)

IN VIVO BIOTINYLATION/EXPRESSION/PROTEIN PURIFICATION PROTOCOL:

Transformation

- 1) Thaw 1 tube of **BirA-transformed Chemically Competent *E. coli* cells** on ice for each transformation. *Note: to increase transformation efficiency, keep cells chilled at all times.*
- 2) Add 1-50 ng of DNA containing the gene of interest into the tube containing **BirA-transformed Chemically Competent *E. coli* cells** in a volume no greater than 10 μ l per 60 μ l of cells. Quickly flick the tube several times to ensure even distribution of the DNA. *Note: it may be advantageous to set up a control to determine transformation efficiency: add 1-10 μ l of a control plasmid to another tube of BirA-transformed Chemically Competent *E. coli* cells.*
- 3) Immediately place tube(s) on ice for 10 minutes.

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- 4) Heat-shock the cells by transferring to a pre-warmed water bath at 42°C for 30 seconds. Do NOT shake the tube.
- 5) Immediately place tubes on ice for 2 minutes.
- 6) Add 900 µl of pre-warmed (37°C) SOC or LB medium to the cells and incubate at 37°C with gentle shaking (225 rpm) for 1 hour.
- 7) Plate 100-200 µl of transformed cells onto plates containing streptomycin or spectinomycin and the appropriate antibiotic for the expression vector. The amount 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.
- 8) Place plates in 37°C incubator for 14-48 hours.

BirA Induction & Expression

- 1) Grow a 50 ml culture of cells in SOC or LB medium containing either 100 µg/ml of streptomycin or 100 µg/ml of spectinomycin overnight. In the morning, pour the entire contents of the tube into 1 L of culture medium with 100 µg/ml of streptomycin or spectinomycin.
- 2) 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.
- 3) Allow culture to incubate overnight at 25°C on a shaker.

Cell Lysis and protein purification

- 1) Dilute **10x lysis buffer** 10 fold to 1x lysis buffer with ice-cold distilled water.
- 2) Collect cell pellet by centrifugation at 6,000 rpm for 10 min, and gently remove the supernatant (culture medium).

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- 3) Wash the cell pellet with 50 ml of PBS, and collect cell pellet again by centrifugation at 6,000 rpm for 10 min.
- 4) Resuspend cell pellet with >3x volume of Lysis Buffer (usually 30–50 ml per L of culture).
- 5) Add protease inhibitors and **1000x DTT** (to final 1x) into lysis buffer.
- 6) Lyse cells by sonicator or cell disruptor or freeze-&-thaw, and remove cell debris by centrifugation at 10,000 xg for 30 min at 4°C.
- 7) Purify protein of interest (biotinylated protein) using appropriate affinity chromatography. If the protein contains additional affinity tags (GST, His, FLAG), we recommend purifying first by the affinity tag, then purify using the AviTag.

QUALITY CONTROL:

4-20% SDS-PAGE

Streptavidin:

Lane 1:

RAF1, + biotin

Lane 2:

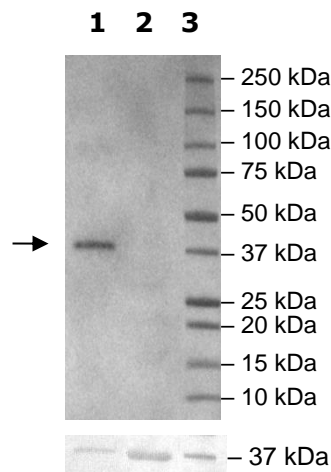
RAF1, - biotin

Lane 3:

Protein Marker

MW: 39 kDa

Ponceau stain:



Biotinylation was confirmed using the *E. coli in vivo* Biotinylation Kit, cat. # xxxxx, and a RAF1-GST-AviTag™ expression construct 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.

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RELATED PRODUCTS

BirA-transformed Chemically Competent *E. coli* cells
BirA, GST-tag
BirA, His-FLAG-tags (*E. coli*-derived)
BirA, His-FLAG-tags (Sf9-derived)

CATALOG #

SIZE

27462 10 vials
70031 100 µg
70030 100 µg
70032 50 µg

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