Recombinant *E. coli* BirA His-tag

Catalog Number: 10464-BA

DESCRIPTION

Source	*E. coli*-derived *e. coli* BirA protein Lys2-Lys321, with an N-terminal Met & 6-His tag Accession # NP_418404.1
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	30-38 kDa, under reducing conditions
Activity	Measured by its ability to generate pyrophosphate from the biotinylation reaction. The pyrophosphate is subsequently hydrolyzed using Recombinant Yeast Inorganic Pyrophosphatase/PPA1 (ryPPA1) (Catalog # 8088-PP). The specific activity is >10.0 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl, TCEP, EDTA and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 150 mM NaCl, pH 7.5
- Recombinant *E. coli* BirA His-tag (rEBirA) (Catalog # 10464-BA)
- Recombinant Yeast Inorganic Pyrophosphatase/PPA1 (ryPPA1) (Catalog # 8088-PP)
- Recombinant Human Tumor Necrosis Factor Receptor Superfamily Member 4 (rhOX40)
- Magnesium Chloride (Sigma, Catalog # M8266), 1 M stock in deionized water
- Biotin (Thermo, Catalog # 29129), 10 mM stock in 5% (v/v) DMSO
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 400 mM stock in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 M Phosphate Standard by adding 10 µL of the 1 M Phosphate Standard to 990 µL of deionized water for a 10 mM stock. Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
2. Continue standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing the 50 µL of Assay Buffer.
4. Prepare reaction mixture containing 2 mM ATP, 20 mM MgCl2, 800 µM Biotin, 280 µg/mL rhOX40, and 8 µg/mL ryPPA1 in Assay Buffer.
5. Dilute rEBirA to 40 µg/mL in Assay Buffer.
6. Load 25 µL of the 40 µg/mL rEBirA into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Load 25 µL of the reaction mixture to wells, excluding the standard curve.
8. Seal plate and incubate at room temperature for 10 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of the deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate at room temperature for 20 minutes.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg) x 2}}$$

*Derived from the phosphate standard curve using linear fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rEBirA: 1 µg
- ryPPA1: 0.2 µg
- ATP: 1 mM
- rhOX40: 7 µg
- MgCl2: 10 mM
- Biotin: 400 µM

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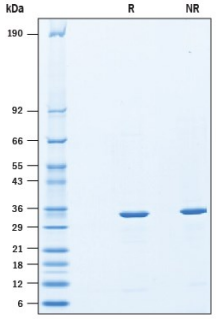
PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

SDS-PAGE



2 µg/lane of Recombinant *E. coli* BirA His-tag (Catalog # 10464-BA) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 30-38 kDa.

BACKGROUND

BirA, the biotin-protein ligase (BPL) of Escherichia coli, is also known as biotin operon repressor, biotin-[acetyl-CoA-carboxylase] ligase, and biotin-[acetyl-CoA-carboxylase] synthetase. BirA, a member of the group II biotin-protein ligase family, contains an N-terminal helix-turn-helix DNA-binding domain, a catalytic core that catalyzes biotinyl 5' adenylate (bio-5'-AMP) synthesis, and a C-terminal domain that plays a role in DNA binding, dimerization, and catalytic function (1). BirA functions both as a DNA-binding protein that represses the biotin biosynthesis operon as well as an enzyme that synthesizes its own corepressor, bio-5'-AMP, an intermediate in biotinylation reactions (1). BirA biotinylates via the lysine side chain of biotin-accepting proteins/peptides, including natural substrate, carboxyl carrier protein (BCCP), and AviTag fusion proteins (1-4). Once biotinylated, (strept)avidin-biotin interactions can be utilized in a wide variety of applications of biochemistry and cell biology, including protein capture, immobilization, multimerizing, and bridging molecules (5).

References:

1. Chapman-Smith, A. and *et al*. (2001) Protein Sci. **10**:2608.
2. Cronan, J. (1989) Cell. **58**:427.
3. Cull, M. and Schatz, P. (2000) Methods Enzymol. **326**:430.
4. Li, Y. and Sousa R. (2012) Protein Expr. Purif. **82**:162.
5. Fairhead, M. and Howarth, M. (2015) Methods Mol. Biol. **1266**:171.

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