MCLAB
Molecular Cloning Laboratories

User Manual

Version 1.0

Product Name: **BirA Protein**

Cat #: **BBLA-100, BBLA-200, BBLA-OEM**

Description:

BirA (bifunctional biotin-[acetylCoA carboxylase] holoenzyme synthetase/ DNA-binding transcriptional repressor, bio-5'-AMP-binding) is a bifunctional protein that exhibits biotin ligase activity and also acts as the DNA binding transcriptional repressor of the biotin operon. Biotinyl-5'-AMP is the co-repressor and is also synthesized by BirA.

Recombinant BirA is suitable for use in a variety of labeling applications and conditions.

Source

E. coli

Purity

> 95 % as determined by SDS-PAGE

Endotoxin

< 1 EU per µg of the protein as determined by the LAL method

Unit Definition

1 Unit is the amount of enzyme that will biotinylate 1 pmol of peptide substrate in 30 minutes at 30°C in Reaction Buffer with 40 µM peptide substrate* in Reaction Buffer**.

*The peptide substrate used in the enzyme assays was a 27kDa AviTag'd recombinant protein expressed by Pichia pastoris.

** Reaction Buffer: 100 mM ATP, 100 mM MgOAC, 500 µM d-biotin

Biological Activity

≥7,500 Units/µg of Biotin-Protein Ligase (BirA Enzyme)

Storage

Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage.

Protocol

Recombinant BirA is suitable for use in a variety of labeling applications and conditions.

Reaction Conditions
The amount of BirA required per reaction is dependent on the molecular weight of the target protein that contains the avidin tag. In general, per 10 nmol of target substrate to be biotinylated, 2.5 µg BirA is suitable for labeling in a 40 µM reaction. An example reaction is shown below for labeling 100 µg of a 150 kDa antibody (0.67 nmol) at 1 mg/ml. In this case, although the concentration of the substrate is less than 40 µM, efficient labeling can be achieved in a range of 2-8 µM using as little as 0.3 µg of BirA.

Example reaction for labeling 150 kDa antibody with an avidin tag:

100 µl antibody (1 mg/ml)
20 µl 0.5M Bicine pH 8.3
20 µl 10X Reaction Buffer (100 mM ATP, 100 mM MgOAC, 500 µM d-biotin)
0.5 µg (1.5 mg/mL BirA)
59.5 µl ddH_20
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Total: 200 µl

Incubate at 30 degrees for 2 hrs. Room temperature reactions are possible if the molarity of the substrate is sufficiently high. Increasing the concentration of BirA enzyme can also be employed to accelerate the reaction.