

AP09093SU-N**Polyclonal Antibody to Biotin Ligase Tag / BLT - Serum**

Alternate names:	Biotin Ligase Epitoe Tag, Biotin-[acetyl-CoA-carboxylase] synthetase, BirA, bioR, dhbB
Quantity:	0.1 ml
Concentration:	70 mg/ml (by Refractometry)
Background:	In <i>Escherichia coli</i> , transfer of biotin to the epsilon amino group of a specific lysine residue of the biotin carboxyl carrier protein (BCCP) is accomplished by the biotin holoenzyme synthetase BirA. The minimal substrate requirement of BirA-catalyzed biotinylation is a 14-residue peptide known as the biotin ligase tag (BLT). This antibody can be used to detect BLT in recombinant expression systems.
Host / Isotype:	Rabbit / IgG
Immunogen:	GLNDIFEAQKIEWH (Minimal BirA substrate; Biotin is added to the sequence at the Lysine) Genename: BirA
Format:	State: Liquid (sterile filtered) Serum Buffer System: 0.02M Potassium Phosphate, 0.15M Sodium Chloride, pH 7.2 Preservatives: 0.01% (w/v) Sodium Azide Stabilizers: None
Applications:	ELISA: 1/5,000. Western blot: 1/5,000. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antiserum detects the minimal peptide substrate (GLNDIFEAQKIEWH) for the <i>E. coli</i> Biotin ligase tag (BLT of BirA). This peptide is also called the Biotin Acceptor Peptide (BAP) of the Biotin Carboxyl Carrier Protein (BCCP) subunit of acetyl CoA carboxylase of <i>E. coli</i> . Western blot analysis of <i>B. subtilis</i> strains producing either (BAP-tagged) SP0IIAH (XWB49) or (BAP-tagged) Sp0IIQ (XWB46) showed strong and specific reactivity to the antisera. Minor background bands of unknown identity may be observed. The antisera will recognize the BAP/BLT tag irrespective of the whether the tag is biotinylated (data not shown). The peptide is a substrate for Biotin ligase, which places a Biotin moiety on the central lysine of the peptide when it interacts with BirA (the Biotin ligase). When proteins contain this sequence tag, they can be very efficiently pulled down after biotinylation using streptavidin beads.
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
General Readings:	1. Schatz PJ. Use of peptide libraries to map the substrate specificity of a peptide-modifying enzyme: a 13 residue consensus peptide specifies biotinylation in <i>Escherichia coli</i> . <i>Biotechnology (N Y)</i> . 1993 Oct;11(10):1138-43. PubMed PMID:

7764094.

2. Beckett D, Kovaleva E, Schatz PJ. A minimal peptide substrate in biotin holoenzyme synthetase-catalyzed biotinylation. *Protein Sci.* 1999 Apr;8(4):921-9. PubMed PMID: 10211839.

Pictures:

Western blot using Anti-Biotin Ligase EPI TOPE TAG antibody shows detection of the BLT Ligase Target in lysates of whole cell *Bacillus subtilis* strains producing either BAP-tagged (Biotin-Acceptor Peptidetagged) SpoIIAH (XWB49) (~ 25 kDa) or BAP-tagged SpoIIQ (XWB46) (~33 kDa). These proteins regulate transcription in *Bacillus subtilis*. The identity of the lower molecular weight bands in the last lane is unknown. Personal communication, J. Meisner, C. Moran, Jr., J. Boss, Emory University School of Medicine, Atlanta, GA.

