



BirA Biotin-Protein Ligase Kit (BirA-500)

Enzyme Specifications

The BirA biotin-protein ligase adds d-biotin covalently to biotin-acceptor peptides/proteins via an ATP intermediate (biotinyl 5'-adenylate) in a highly efficient and targeted manner. The protein is encoded by the *birA* gene and is purified using traditional methods. The molecular weight of the BirA biotin-protein ligase is 33,500 Daltons.

Other names for this enzyme include: biotin ligase; biotin operon repressor protein; birA; biotin holoenzyme synthetase; biotin-[acetyl-CoA carboxylase] synthetase; biotin-[acetyl-CoA-carboxylase] ligase; biotin-[acetyl-CoA carboxylase] synthetase; acetyl CoA holocarboxylase synthetase; acetyl CoA holocarboxylase synthetase; biotin:apocarboxylase ligase; biotin holoenzyme synthetase; HCS.

Source: *E. coli*

Enzyme Storage buffer: 50mM imidazole (pH 6.8), 50mM NaCl, 10% glycerol, 5mM dithiothreitol (DTT).

Storage conditions: The BirA-500 biotin-protein ligase kit arrives on dry ice (or, in rare cases, ice brix) via overnight delivery and should be immediately stored at -80°C. After an enzyme vial is thawed it should be stored at 4°C if it is to be re-used in the near future. For long-term storage keep at -80°C. A vial of thawed enzyme can be re-frozen by dropping into liquid nitrogen before storing again at -80°C.

The other kit components, BiomixA, BiomixB and BIO-200, can be stored long term at -20°C. For short term storage 4°C is fine for the BiomixA and BIO-200. The BiomixB should always be stored at -20°C or colder and thawed immediately before use. There is no problem in thawing and re-freezing any of these mixtures repeatedly.

Stability: BirA biotin-protein ligase retains >80% of its activity when stored for one month at 4°C. Long term storage should be at -80°C. Refreezing the enzyme is possible but will reduce its activity upon thawing again by 10% each time. Therefore we don't recommend repeated thawing and refreezing cycles with the BirA.

Concentration: 1.0 mg/mL by A280 (Extinction coefficient; 1.349AU at 280nm $\lambda=1\text{mg/mL BirA}$).

Purity: >99% by Coomassie staining.

Activity: a minimum of 5,000 Units/ μg of BirA

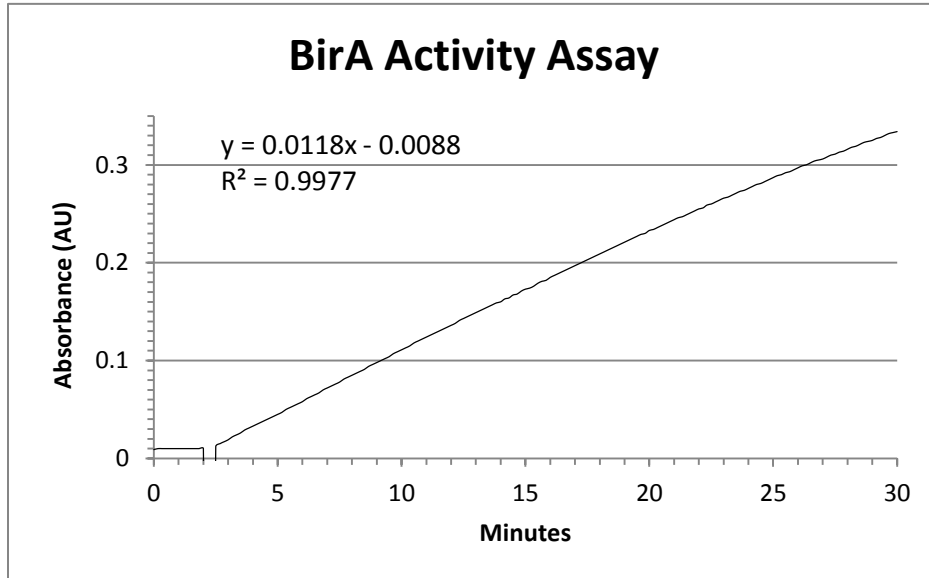
Definition of Activity: One (1) Unit is the amount of enzyme that will biotinylate 1pmol of biotin-acceptor peptide substrate* in 30 minutes at 30°C using the reaction buffers provided and 40 μM peptide substrate final concentration.

* The peptide substrate used in the enzyme assays is a 15-mer variant of sequence #85 identified by P. Schatz (1).

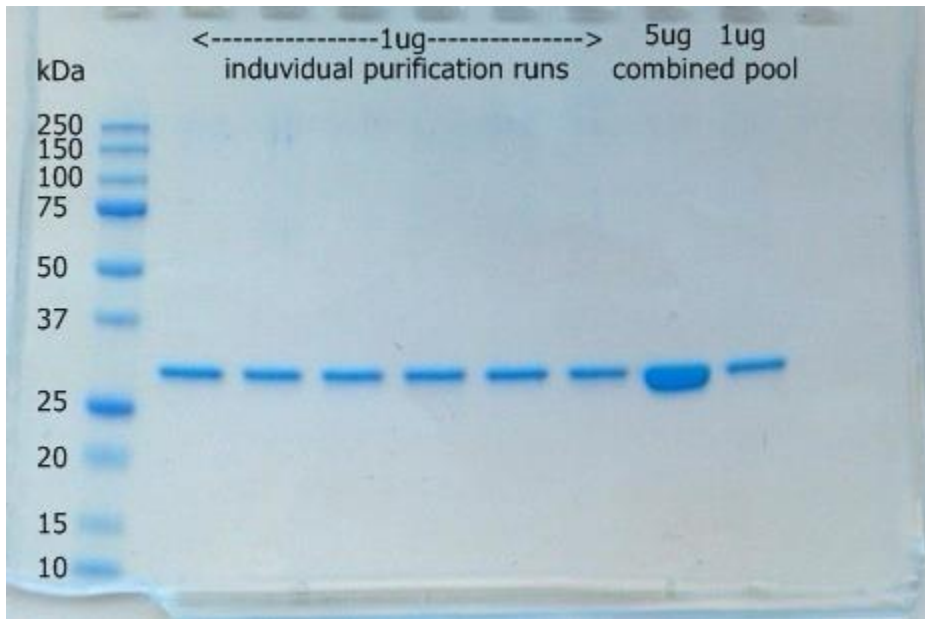
Contaminating proteases: <0.01% as chymotrypsin-like activity.



Typical BirA kinetics activity assay profile:



Representative BirA appearance on 12% polyacrilamide SDS-PAGE gel*:



*photo edited for brightness and contrast. ©Avidity LLC, 2014. All rights reserved.

References: (1) Schatz, P. (1993) Biotechnology 11, 1138-1143