Specifications for Bulk BirA-RT Kit

Lyophilized biotin-protein ligase reaction kit

Description: Biotin-protein ligase (EC 6.3.4.15) activates biotin to form biotinyl-5'-adenylate and transfers biotin, efficiently and specifically, to the lysine residue in the AviTag™ sequence GLNDIFEAQKIEWHE.

Source: Overexpressed in *E. coli* B-strain.

Storage conditions: The kit arrives at ambient temperature and should immediately be stored at 4 °C. The kit may be stored as received for one year at 4 °C. The enzyme arrives lyophilized in ten 0.5-mL skirted tubes (30 µg per tube). Once reconstituted, the enzyme can be stored for up to two weeks at 4 °C without loss of activity. Long term storage after reconstituting the enzyme requires flash freezing the tube in liquid nitrogen and then storing at -80 °C. Once reconstituted, the enzyme may be stored for up to a year at -80 °C without significant loss of activity.

Stability: Reconstituted BirA will retain >99% of its activity for up to 4 hours when left at room temperature. At 4 °C, the enzyme will retain >99% of its activity for up to 2 weeks and >90% for up to 3 months. At -80 °C, the enzyme will remain >99% active for at least 1 year.

Purity: >99% by Coomassie-stained polyacrylamide gel assay.

Activity: >8000 units/µg of BirA.

Definition of Activity: One Unit (U) of BirA is the amount of enzyme that will biotinylate 1 pmol of AviTag™-containing peptide substrate in solution at 40 µM within 30 minutes at 30 °C.[1]

Protease activity: No detectable activity.

References:

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

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Instructions for Use

Components Provided

- BirA enzyme: 10 vials; 30 µg each of lyophilized BirA enzyme; 300 µg total
- Resuspension Buffer: 1 vial; 500 µL BirA resuspension buffer
- SuperMix: 1 vial; 10 mL 10x SuperMix buffer
- ATP: 1 vial; 550 mg under argon gas
- Additional empty vials: 10 labeled 2-mL tubes for SuperMix/ATP storage

Component Preparation

BirA

Reconstitute lyophilized BirA with the storage buffer provided. This BirA is highly soluble in aqueous buffers. The recommended volume for reconstitution is 30 µL for a final enzyme concentration of 1 mg/mL. Care must be taken to make sure all of the lyophilized BirA gets contacted by the buffer. Pipette the buffer up and down the walls of the tube to ensure all the BirA is in solution. Once in solution use immediately or follow the storage conditions outlined above.

SuperMix

SuperMix requires ATP to be added before use. We provide the ATP under argon gas in a separate tube for quick mixing. Add the ATP directly to the tube containing 10 mL of SuperMix. Mix well until all the ATP goes into solution. If the ATP does not dissolve completely within a few minutes, add 10 N NaOH dropwise with mixing until the ATP dissolves and the solution is clear. Once mixed completely, aliquot the SuperMix/ATP into the provided labeled storage tubes, 1 mL per tube. Immediately freeze and store the aliquoted SuperMix at -80 °C until use. Thawed SuperMix/ATP should be kept on ice during use.

Example Reaction

For this example, our reaction will be done at room temperature. We will be using Maltose-Binding Protein (MBP) conjugated with the AviTag™. The MW of the MBP-Avi is 43,929 Da. For simplicity we will round up to 44 kDa. We will use our recommended reaction conditions of 5 µg of BirA per 50 nmol of AviTag™-containing substrate at 100 µM.

- 1 nmol of a 44 kDa protein = 44 µg; therefore 50 nmol = 2200 µg.

For 100 µM substrate reaction conditions, the 2200 µg of MBP-A needs to be in a final reaction volume of 0.5 mL. 100 µM = 100 µmol/L = 100 nmol/mL; or 50 nmol/0.5 mL.
Therefore, our reaction would look as follows:

- 5 µg of BirA (the volume of BirA added is low enough not to need accounting)
- 50 µL of SuperMix (10x)
- 450 µL buffer* containing 2.2 mg of MBP-A
- 0.5 mL final volumecontaining 50 nmol of MBP-A at 100 µM with 5 µg of BirA

*favorable substrate buffers are discussed below.

In the above example the MBP-A was at 4.4 mg/mL final concentration. If your substrate is not this concentrated the reaction can be adjusted to suit your situation.

BirA quantity in a reaction follows the inverse square rule for reaction rate when volume and substrate concentration are held constant.

- Adding half the amount of BirA increases the time to complete the reaction by 4x.
- Adding twice the amount of BirA decreases the reaction time by half.

BirA can tolerate long reaction times at room temperature so your desired reaction time needs to be based upon the tolerance of your substrate for being at room temperature. The reaction may be carried out at 4 °C without issue but will take substantially longer to complete.

Figures

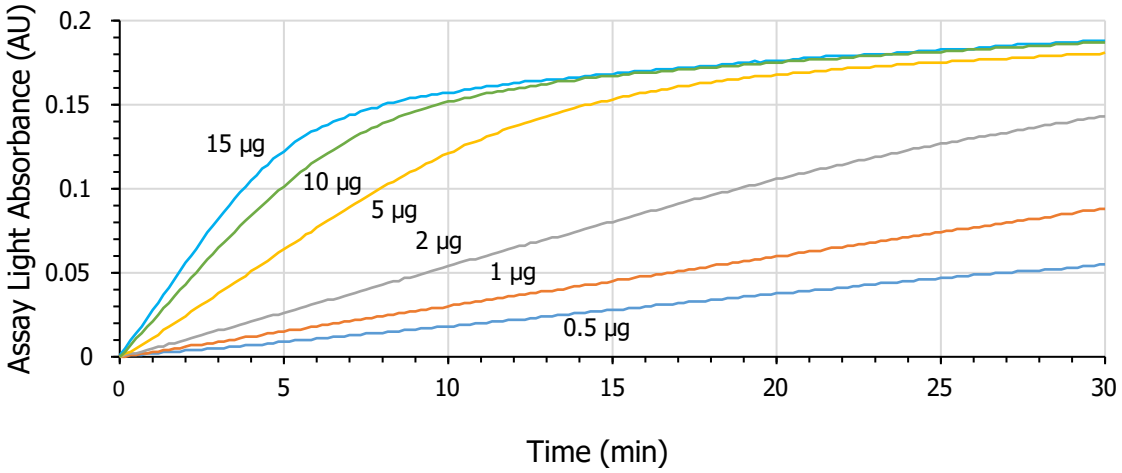

Figure 1. Effect of BirA quantity on reaction rate (constant volume and substrate quantity).

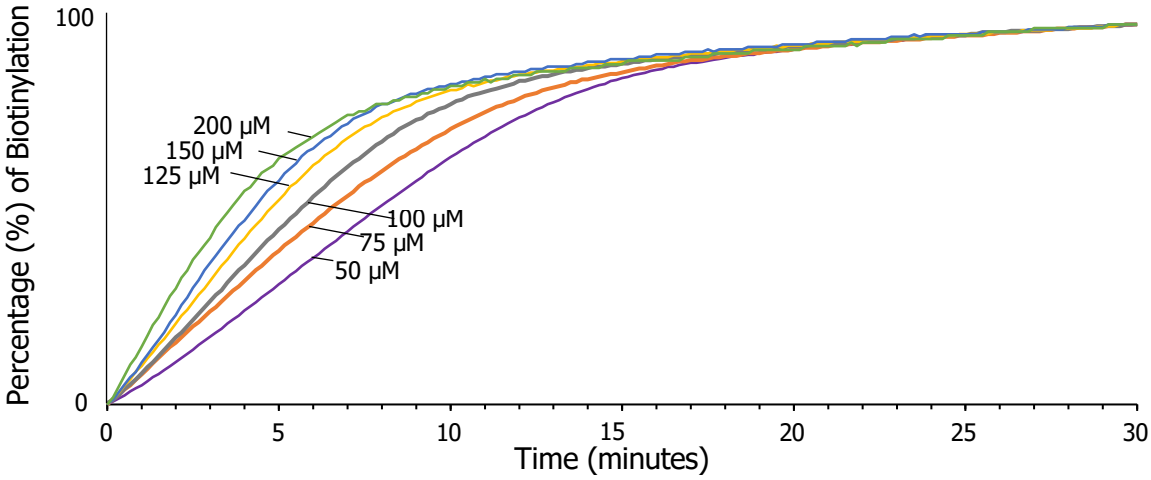

Figure 2. Effect of substrate concentration on reaction rate (constant volume and BirA quantity).

Compatible Reaction Buffers

The SuperMix is a 10x solution that needs to be diluted 1:10 in the reaction buffer which contains your AviTag™-containing protein substrate. The BirA will biotinylate well under a variety of buffer conditions. But a few buffer conditions should be avoided as they slow the reaction considerably.

- NaCl: concentrations of NaCl over 100 mM should be avoided. Here, less is better. If a salt is needed to maintain the solubility or stability of your target substrate we recommend using potassium glutamate (MPG). BirA tolerates very high concentrations of MPG.
- Glycerol: concentrations over 5% should be avoided.
- Ammonium Sulfate: concentrations over 50 mM should be avoided.
- pH: >8.8 and <6.5 should be avoided.

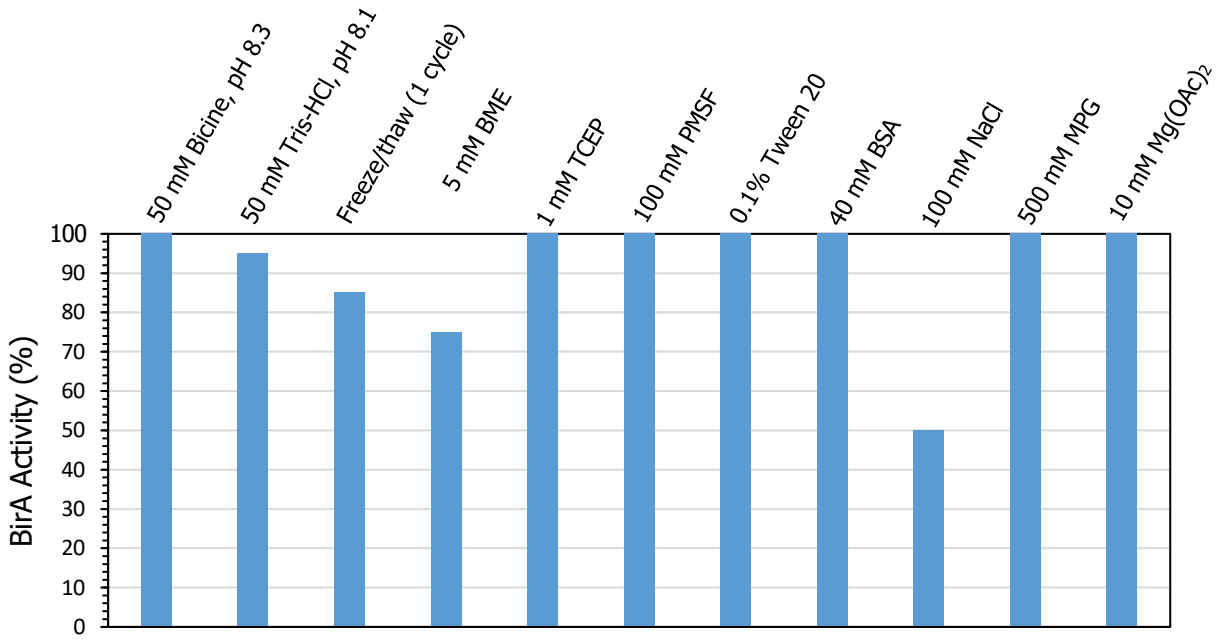

Figure 3. Percentage of normal BirA activity (used as directed, no additives) in various buffer conditions.

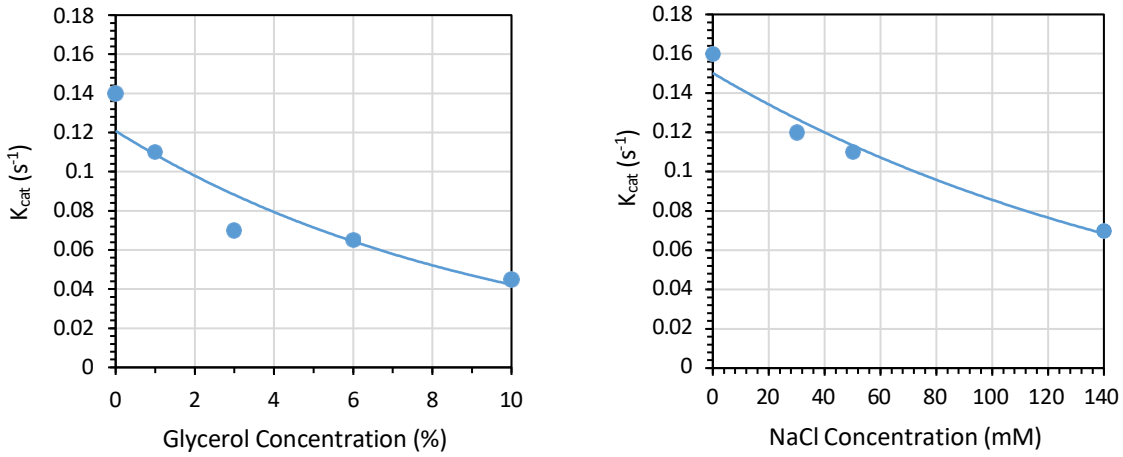

Figure 4. Effects of glycerol and NaCl on biotinylation reaction rate.

Removal of Unreacted Biotin

Removing the unreacted biotin upon the completion of the reaction is critical in some cases, as remaining free biotin will compete with your biotinylated protein for biotin-binding sites on avidin/streptavidin. There are several ways to remove the free biotin from the completed reaction.

- Dialysis: Dialysis is a tried-and-true method, but works best when the sample volume is small so the sample can be dialyzed against large volumes of buffer. We like Pierce Slide-A-Lyzers for convenience. Sample should be dialyzed for at least 2 hours at 1:500 sample/buffer with at least four changes of buffer.
- Desalting column: Desalting columns are quick and convenient, but work best with small volumes (≤1 mL or as recommended in product instructions). We like the Zeba™ Spin Desalting Columns, again from Pierce, for convenience. But many other desalting columns work well. We recommend at least 2 sequential passes through the columns which should reduce free biotin by about 400x.
- Affinity or ion exchange: This is the best method for removing free biotin, especially if the sample volumes are large. Binding the biotinylated protein to a resin column allows the bound protein to be washed free of the remaining unreacted biotin (a wash of 5 to 10 column volumes is recommend). The protein is then eluted using the appropriate elution buffer for the resin. Many of our customers use IMAC to remove unreacted biotin from His-tagged proteins, and this has reportedly worked well for them.

Related Products

BIS-300: a positive and negative control protein kit. The BIS-300 Kit contains a fully biotinylated MBP- AviTag™ fusion protein standard and an unbiotinylated MBP-AviTag™ fusion protein that may be used alongside your BirA biotin-protein ligase reactions for comparison of "extent-of-biotinylation" via SDS- PAGE gel analysis, in Western blot analysis, or in ELISA formats.

Technical Help

Questions concerning this product or the procedure outlined is this packaging insert may be directed to info@avidity.com, or call us at +1 720 859 6111 (+1 877 333 6063 toll free).