Basic INSTRUCTIONS FOR USE

Components provided:

- BirA biotin-protein ligase [1mg/ml (BirA500) or 3mg/ml (Bulk BirA)]
- BiomixA (10X concentration: 0.5M bicine buffer, pH 8.3)
- BiomixB (10X concentration: 100mM ATP, 100mM MgOAc, 500µM d-biotin)
- BIO200 (d-biotin; 10X concentration: 500µM)

The final reaction mixture should optimally contain:

- 1X BiomixA
- 1X BiomixB
- Substrate peptide (protein) containing AviTag™ (for concentration see discussion below)
- BirA enzyme (for amount see discussion below)
- BIO200 as necessary (see note below).

To ensure a rapid rate of biotinylation, it is recommended that the AviTag'd substrate be as close as possible to 40µM in the final reaction mix. The lower the substrate concentration in the reaction mix, the longer it will take for biotinylation to complete. For example, a substrate at 40µM may be completely biotinylated within 30-40 minutes, but at 4µM it will take more than 5 hours using the same amount of BirA enzyme. To complete the biotinylation reaction at 4µM in under an hour (i.e. 10 times faster), it is necessary to add 10 times more enzyme to the reaction mix. As it is, the amount of BirA enzyme added to the reaction mix may need to be varied to achieve biotinylation within an acceptable time-frame (see below).

The BirA enzyme has been tested and shown to be free from measurable protease contamination. Longer incubation times do carry the risk that your target protein may be proteolyzed from proteases present in small amounts from target protein purification, and we recommend using the shortest incubation time that fully biotinylates the target protein. Longer incubation times may be alos problematic the target protein from ATP. ATP in solution will hydrolyze with time, lowering the available ATP for the biotinylation reaction. Therefore completing the reaction quickly will be beneficial to achieving maximal biotinylation.

Biomix B contains ATP that might subject to hydrolysis if not handled correctly. Biomix B is the most labile component of the BirA reaction kit, and the Biomix B should be thawed on ice and immediately re-frozen after addition to the BirA reaction.

Typically, for every 10 nmol of substrate (at 40µM), we recommend 2.5µg of BirA enzyme to complete the biotinylation in 30 - 40 minutes at 30°C or about 1 hour at room temperature. Reactions can be performed at 4°C but will require more time, overnight if it is convenient. This is acceptable because the ATP hydrolyzes more slowly at 4°C. Also, additional ATP in solution may be added to the reaction if there is a question of incomplete biotinylation due to ATP degradation. The other reactants are more than stabile enough to continue the biotinylation if this is the case.

The BiomixB has been optimized for the biotinylation of substrates at concentrations of no more than 40µM. If it is desired to biotinylate substrates at concentrations of 40-80µM, then it is necessary to supplement the reaction mix with additional biotin. **The BIO200** (500µM d-biotin) has been included to supplement reaction mixes of up to 80µM. For substrate concentrations above 80µM, please contact our Technical Support at (720) 859 6111 or Toll-Free (877) 333 6063.

Example calculation for determining the amount of BirA needed for your reaction:

For this example we will assume we are using a peptide substrate with AviTag™ of 15 kDa MW (note: the AviTag™ is 1.829 kDa MW).

1 nmol of a 15 kDa protein = 15µg; therefore 10 nmol = 150µg (2.5µg of BirA is recommended per 10 nmol).
For a 1mg/mL solution of the substrate, 0.5mL would contain 500µg of that substrate.

- 500µg substrate/(150µg substrate/10 nmol) = 33.3 nmol of substrate to be biotinylated
- 33.3 nmol of substrate/(10 nmol of substrate/2.5µg BirA) = 8.325µg BirA

For best efficiency the substrate needs to be at 40µM concentration. In our example we have currently 0.5mL at 1mg/mL.

- 1mg/mL = 1000µg/mL = 66.6 nmol/mL = 66.6 µmol/L = 66.6µM.

We need to dilute the reaction solution to 40µM final.

- (66.6µM/40µM)(X/0.5mL); X = 0.83mL

So the final reaction mix would need to be brought up to 0.83mL with BiomixA (10X), BiomixB (10X), BirA and water (or your buffer of choice, see precautions below).

- 500µL of 66.6µM substrate + 83µL BiomixA + 83µL BiomixB + (164µL – XµL of BirA for 8.325µg*) water + XµL BirA = 0.83mL final reaction volume at 40 µM substrate concentration. (*BirA is offered at 1mg/ml or 3mg/mL concentrations).

Examples of BirA quantity per substrate amount for three different

Substrate MW	10 nmol substrate	nmol of substrate in 0.5mL of 1mg/mL solution (500µg)	Amount of BirA needed for 500µg*	Reaction volume at 40µM
15 kDa	150 µg	33.3 nmol/500µg	8.325 µg	500µg/0.83 mL
75 kDa	750 µg	6.67 nmol/500µg	1.67 µg	500µg/0.17 mL
225 kDa	2250 µg	2.2 nmol/500µg	0.55 µg	500µg/0.06 mL

(*at 2.5µg BirA per 10 nmol AviTag'd substrate)

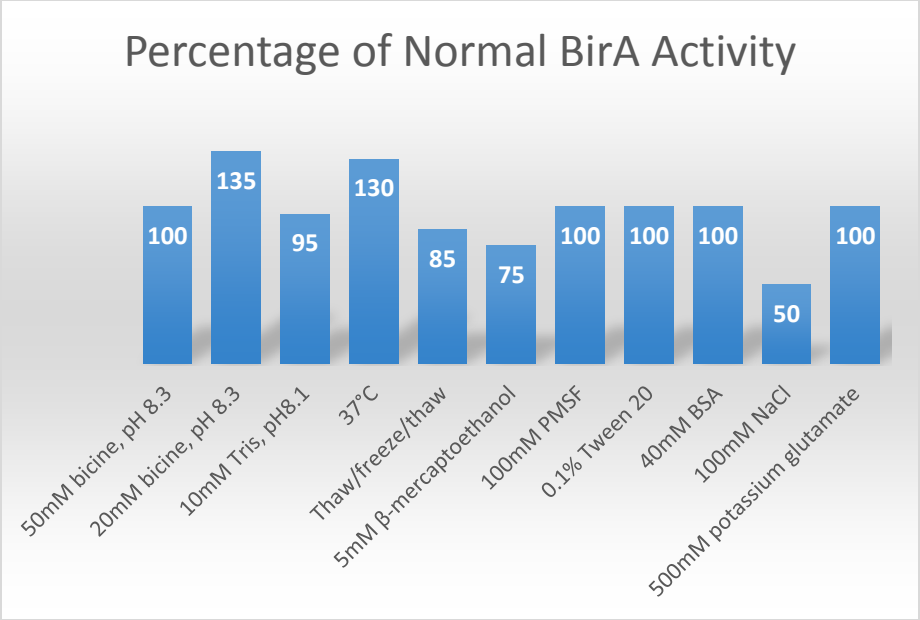
Note from the table above that it may be necessary to concentrate your substrate to meet the 40µM criteria.

Buffer conditions that affect biotin ligase:

It should be noted that various reagents commonly present in biological buffers will reduce the activity of BirA enzyme. These include NaCl, glycerol and ammonium sulfate among others (see chart below). Consequently, the concentration of these reagents in the substrate solution should be minimized. We recommend that, if possible, the substrate be suspended in 50 mM bicine, pH 8.3, but 10 mM Tris-HCl, pH 8 can be substituted.

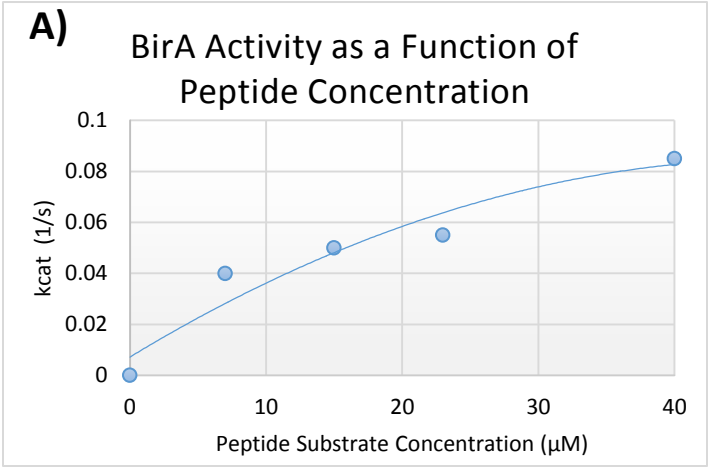
If the protein substrate needs "salts" to maintain stability or activity, we suggest substituting potassium glutamate for sodium chloride. Potassium glutamate is compatible with biotin ligase at higher concentrations and can provide an ionic environment that may stabilize proteins.

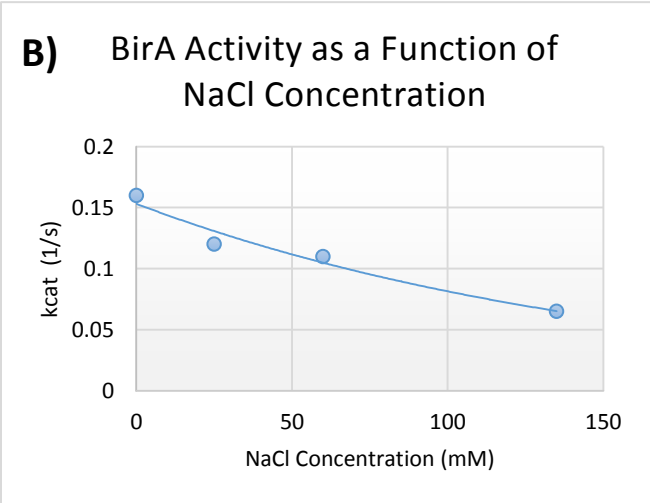
Reaction conditions for BirA biotin ligase

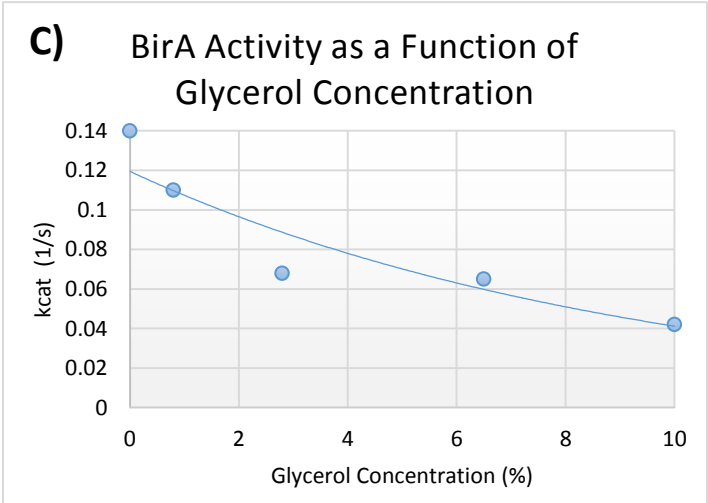


Biotin ligase (BirA) is showing different activities for various buffer conditions.

Following graphs are showing how the enzyme activity of BirA is dependent on (A) substrate concentration, (B) NaCl concentration and (C) Glycerol concentration.







Related products:

BIS-300 Positive Control Substrate 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 as an extent-of-biotinylation comparison for BirA biotin-protein ligase reactions, for SDS-PAGE gel analysis or in Western blot analysis.