Basic INSTRUCTIONS FOR USE

***Components provided*:**

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

***The final reaction will contain the following*:**

- 1/10 BiomixA (other buffers may be used, see below)
- 1/10 BiomixB
- 8/10 dH_2O – x (where x = substrate volume)
- Substrate with AviTag™ (volume depends on substrate concentration)
- BirA enzyme (2 µg/100 µg substrate present in the reaction)

An example 1 mL reaction mix might look like this*:

- 700 µL dH_2O
- 100 µL BiomixA
- 100 µL BiomixB
- 100 µL AviTag'd substrate (100 µg at 1 mg/mL for this example)
- 2 µg BirA

*Your reaction volume can be anything that is convenient for you as long as the ratios of the reactants remain consistent.

Please note: The optimal reaction conditions for every substrate will be slightly different. The example reaction mix that we provide above is good to get you started and will yield excellent results. Some adjustments on your part, such as BirA amount, buffer conditions, reaction time or temperature, may need to be made in order to maximize BirA performance. But rest assured that the starting conditions above will get you where you want to be.

BirA is most active at 37°C, but BirA also performs well at lower reaction temperatures: At 30°C, under optimal conditions, an hour may yield ≥95% biotinylation. Reactions may be done at room temperature and should be allowed to go for at least 2 hours. They may also be done overnight at 4°C.

Increasing the amount of BirA enzyme in the reaction can expedite the reaction, provided that all essential components are available. We recommend starting at 2 µg of BirA per 100 µg of AviTag'd substrate. These reaction conditions serve as general guidelines to determine the optimal conditions for your specific requirements.

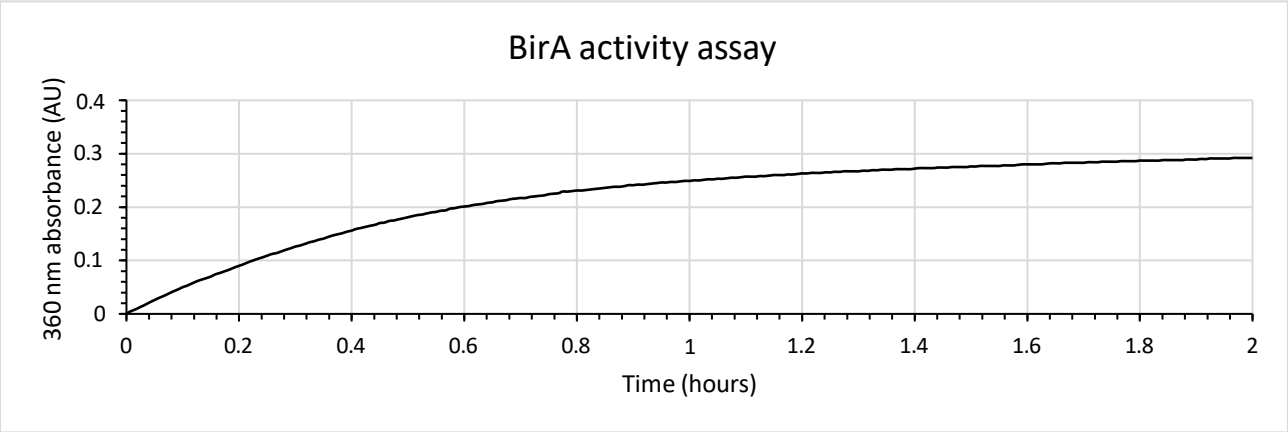
Buffer conditions that affect biotin ligase:

Reagents commonly found in biological buffers can reduce the activity of the BirA enzyme. These include NaCl (specifically Cl^-), glycerol, and ammonium sulfate, among others. The concentration of these inhibitory reagents in the substrate solution should be minimized. We recommend suspending the substrate in 50 mM bicine, pH 8.3 (see BiomixA). Other buffers (Tris pH 7.5-8.3, HEPES 7.5-8.3,) may be substituted.

If the protein substrate requires high salt conditions for stability or activity, consider substituting potassium glutamate for sodium chloride. Potassium glutamate is compatible with biotin ligase at higher concentrations and can provide an ionic environment that stabilizes proteins.

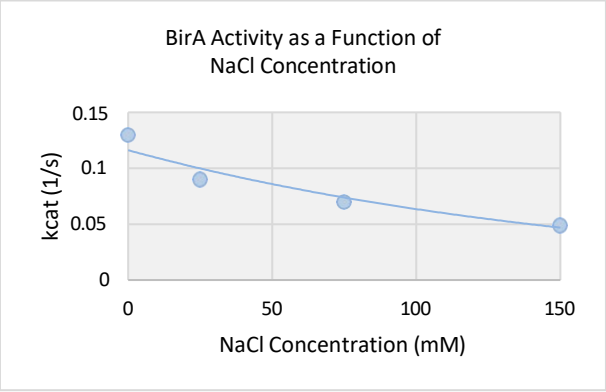
BiomixA is a bicine reaction buffer, pH 8.3, which we use and recommend in most cases because it works well with BirA.

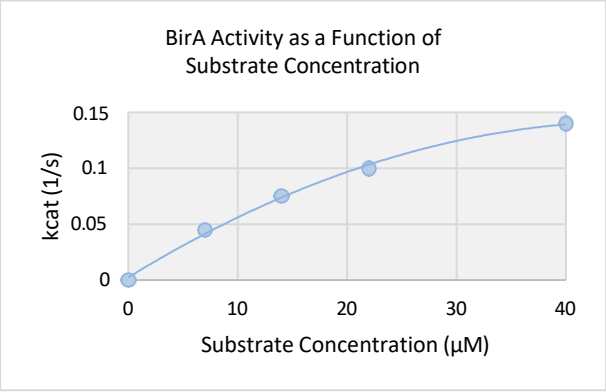
BiomixB contains ATP, which may be subject to hydrolysis. As the most labile component of the BirA reaction kit, BiomixB should be thawed on ice and immediately refrozen after use. Prolonged incubation at room temperature can lead to ATP hydrolysis, reducing the available ATP for the biotinylation reaction.

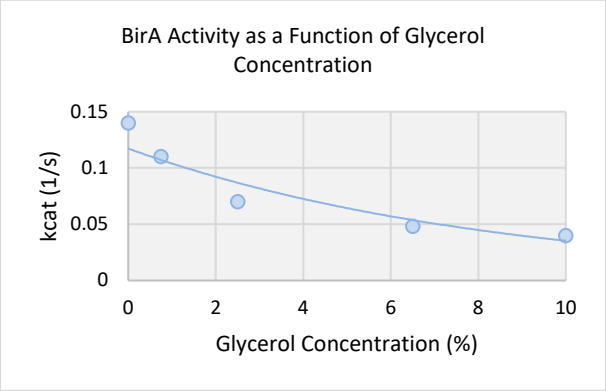


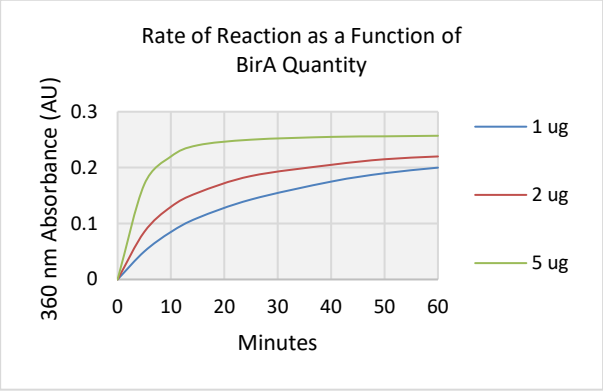
The conditions illustrated in the graph above show the rate-of-reaction for a particular 16-amino acid substrate (the actual AviTag sequence with a C-terminal cysteine) that we use to measure BirA activity in-house. These conditions have been fine-tuned for rapid, reproducible biotinylation of this particular peptide and form the basis of our reaction protocol. However, it is important to note that the optimal conditions may vary for other AviTag'd substrates. Nevertheless, these general conditions should yield satisfactory biotinylation levels.

Appendices:

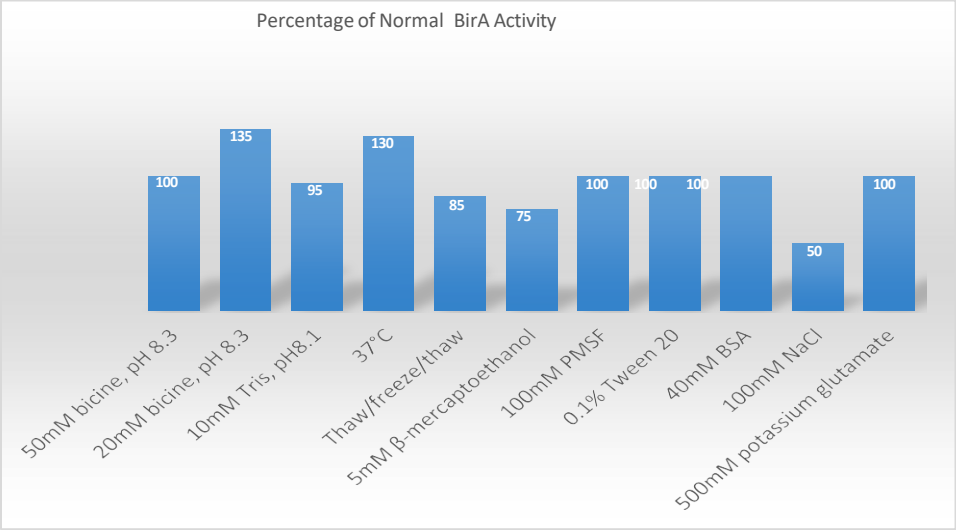








Levels of biotin ligase (BirA) activity in various buffer conditions



Related products:

BIS-300 Positive Control Substrate Kit:

The BIS-300 Kit contains a fully biotinylated MBP-AviTag™ fusion protein (44.1 kDa) standard and an unbiotinylated MBP-AviTag™ fusion protein (43.9 kDa) 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.