AVIDITY

Protocols
Reaction conditions for biotin ligase (BirA enzyme)

Basic INSTRUCTIONS FOR USE

Components provided:

- BirA enzyme- biotin ligase (1 mg/ml or 3 mg/ml)
- Biomix-A (10X concentration: 0.5 M bicine buffer, pH 8.3)
- Biomix-B (10X concentration: 100 mM ATP, 100 mM MgOAc, 500 µM d-biotin)
- Additional d-biotin (10X concentration: 500 uM)

The final reaction mixture should optimally contain:

- 1X Biomix-A
- 1X Biomix-B
- substrate containing AviTag (see concentration discussion)
- BirA enzyme. (see amount discussion below)
- Additional biotin can be added (1X, see note below).
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To ensure a rapid rate of biotinylation, it is recommended that the substrate be as concentrated as possible in the final reaction mix (up to 40 µM). The lower the substrate concentration in the reaction mix, the longer it will take to biotinylate. For example, whereas a substrate at 40 µM may be biotinylated in ~30 min., at 4 µM it will take ~5 hrs using the same amount of birA enzyme. To perform the biotinylation at 4 µM in 30 min. (i.e. 10 times faster), it is necessary to add 10 times more enzyme to the reaction mix.

The amount of birA enzyme to add to the reaction mix may need to be varied to achieve biotinylation within a reasonable time-frame (see below). Typically, for every 10 nmol of substrate (at 40 µM), we recommend 2.5 µg of birA enzyme to complete the biotinylation in 30 - 40 min. at 30°C.

Biomix-A and -B have been optimized for the biotinylation of substrates at concentrations of no more than 40 µM. If it is desired to biotinylate substrate at concentrations of 40-80 µM, then it is necessary to supplement the reaction mix with additional biotin as follows: 1 part Biomix-A, 1 part Biomix-B, 7 parts substrate solution, 1 part supplemental biotin. For substrate concentrations above 80 µM, please contact our Technical Help at (720) 859 6111 or Toll-Free (877) 333 6063.

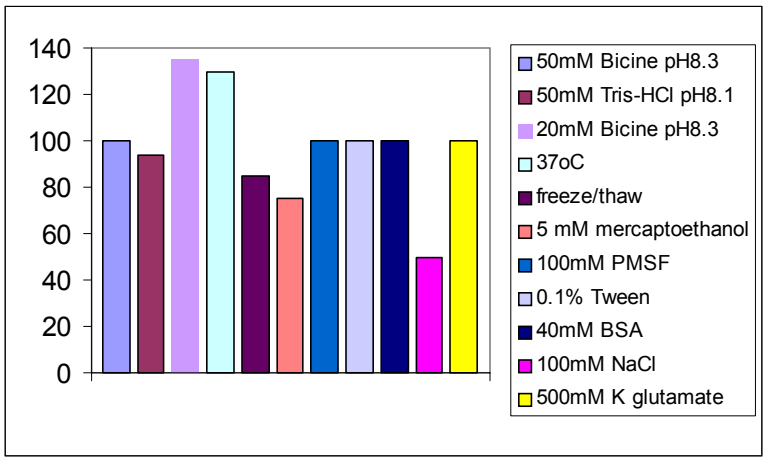
Buffer conditions that affect biotin ligase

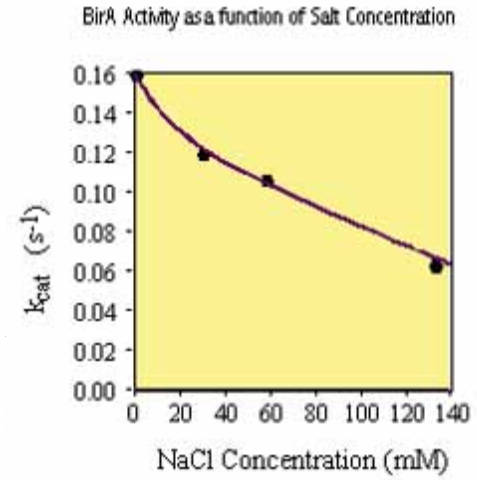
The reaction conditions described above have been optimized for a 15-mer peptide similar to sequence #85 identified by Schatz (1). We have investigated the optimum reaction conditions for substrates in which the biotin peptide tag is attached to a protein, and found that they are identical to the reaction conditions for the peptide substrate.

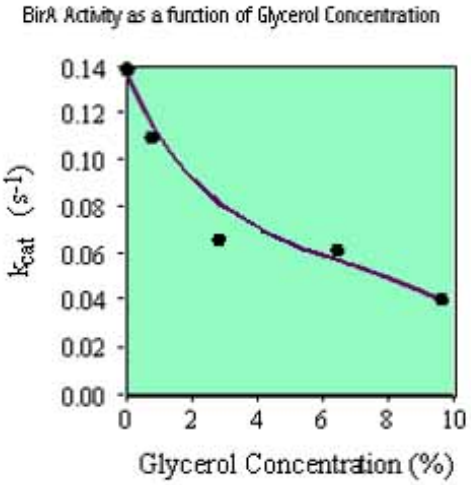
It should be noted that various reagents commonly present in biological buffers will inhibit the activity of birA enzyme. These include NaCl (100 mM), glycerol (5%) and ammonium sulfate (50 mM). Consequently, the concentration of these reagents in the substrate solution should be minimized. We recommend that, if possible, the substrate be added to the reaction mix in 50 mM bicine, pH 8.3, but 10 mM Tris-HCl, pH 8 can be substituted.

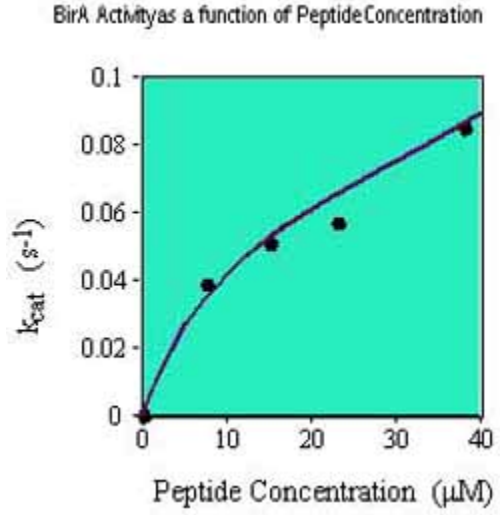
If protein of interest needs "salts" to maintain stability or activity, we suggest substituting Potassium glutamate for Sodium chloride. K Glu is capatible with biotin ligase and can provide an ionic environment that may stabilize proteins.

Avidity sells BIS-300- Positive control substrate kit. The kit contains unbiotinylated Avitag'd maltose binding protein that can be place in experimental buffer conditions for a biotin ligase reaction. The kit also contains fully biotinylated Avitag'd maltose binding protein as a positive control. These proteins can be used as standards for determining the extent of biotinylation of experimental proteins.









For other enzyme conditions- BirArxngraph.doc

Related products

BIS-300 positive and negative control protein kit

BIO-200 Biotin solution

Download the specification sheets:
BirA 500.doc