AVIDITY

Protocols
Biotin ligase reaction in bacterial lysates

Biotin ligase can biotinylate Avitag'd proteins in most bacterial lysates. This allows the Avitag protein to be biotinylated prior to purification or desired use.

Basic INSTRUCTIONS FOR USE

Components needed:

- 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
- BirA enzyme.
- Additional biotin can be added

Typical bacterial lysates allow biotin ligase to biotinylate AviTag as long as the following guidelines are followed:

- NaCl concentration is below 50 mM
- pH of extract is above 7.5
- protein with Avitag is at least 1% of the protein in the extract.
- Extract should not contain ammonium sulfate, B-mercaptoethanol, or glycerol.
- See Purified protocol.doc for more details

Biotin ligase reactions can tolerate low amount of non-ionic detergents. Extracts generated using BPER (Pierce) are compatible.
If the Avitag'd protein is not 1% of the protein and reactions incubated for long times, cellular proteins not Avitag'd will become biotinylated at a small rate (enough to be detected on western blots)