



ProFoldin

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INSTRUCTIONS

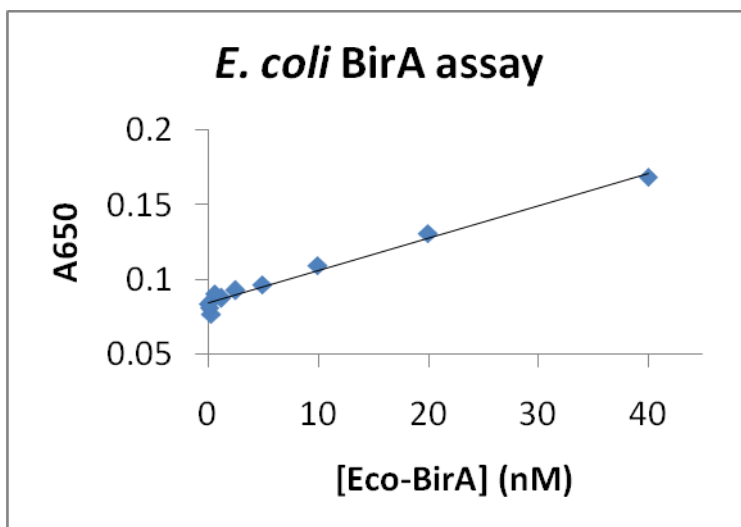
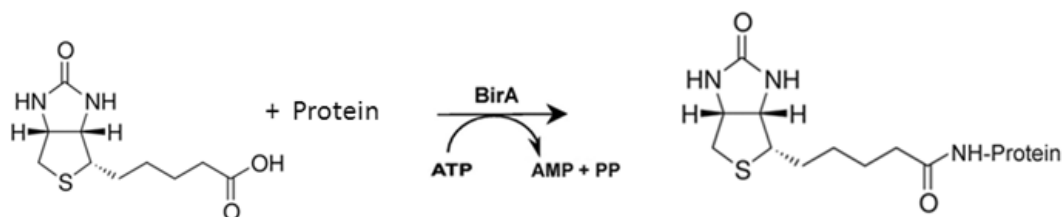
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E. coli Biotin Protein Ligase (BirA)

E. coli Biotin Protein Ligase – for 100 assays

Catalog No. BIRA-100EC

Protein construct:	Wild-type <i>E. coli</i> BirA purified from a bacterial expression system.
MW:	35 kDa
Enzyme concentration:	5 μ M
Enzyme activity assay:	The BirA activity is measured by using the <i>E. coli</i> Biotin Protein Ligase Assay Kit (Catalog No. BPL100K).
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution.



The *E. coli* Biotin Protein Ligase – for 100 assays (Catalog No. BIRA-100EC) contains 33 μ l of 100 x *E. coli* BirA (5 μ M). It is for 100 assays.

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Assay Protocol using *E. coli* Biotin Protein Ligase Assay Kit

The following assay protocol is based on the 384-well plate assay format. The reaction volume is 30 µl and the final assay volume is 75 µl. For 96-well plate assays, the reaction volume is 60 µl and the final assay volume is 150 µl. For detection using a cuvette, the reaction volume is 400 µl and the final assay volume is 1000 µl.

1. Reagent preparation:

For each 10 assay reactions,

- (1) Prepare 297 µl of premix composed of 261 µl of H₂O, 33 µl of 10 x Buffer, 3.3 µl of 100 x *E. coli* BirA and 3.3 µl of 100 x PPase.
- (2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3 µl of 100 x Biotin, 3.3 µl of 100 x hydroxylamine, 3.5 µl of 100 x ATP with 23.1 µl of water.

2. Reaction:

Mix 27 µl of the premix with 3 µl of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at a temperature between 25 °C and 37 °C for 60 min.

3. Detection:

Add 45 µl of the Dye MPA3000 into the 30 µl of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.

Assay Protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at <http://www.profoldin.com/services.html>.

Related Products

E. coli Biotin Protein Ligase Assay Kit

Catalog No. BPL100K

E. coli Biotin Protein Ligase Assay Kit Plus-100

Catalog No. BPL100KE

E. coli Biotin Protein Ligase Assay Kit Plus -500

Catalog No. BPL500KE

ProFoldin offers high throughput assays for various drug targets and enzymes, for more information, please visit www.profoldin.com or send emails to info@profoldin.com.