ProFoldin
10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Phone: (508) 735-2539 **FAX: (508) 845-9258**
www.profoldin.com info@profoldin.com

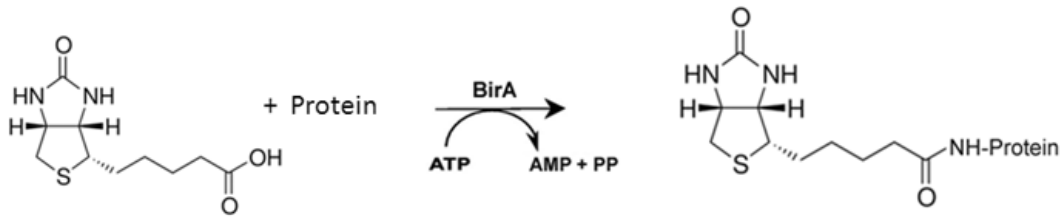
INSTRUCTIONS

ProFoldin
E. coli Biotin Protein Ligase Assay Kits

***E. coli* Biotin Protein Ligase Assay Kit** **Catalog No. BPL100K**
***E. coli* Biotin Protein Ligase Assay Kit Plus-100** **Catalog No. BPL100KE**

INTRODUCTION

Biotin protein ligase (BPL or BirA) is responsible for biotinylation of biotin-dependent proteins. It is an essential enzyme and an attractive target for anti-bacterial drug discovery.



The ***E. coli* Biotin Protein Ligase Assay** is based on measurement of the pyrophosphate generated from the BirA reaction using hydroxylamine in the place of protein as a biotin receptor. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by light absorbance at 650 nm. The assay reactions and detection can be performed by using 384-well or 96-well assay plates. Alternatively, the assay reaction can be carried out in Eppendorf tubes and the signal is measured using a cuvette. The high throughput assay can be used for screening inhibitors of *E.coli* BirA in drug discovery research. It may also be used for characterization of *E.coli* BirA.

The ***E. coli* Biotin Protein Ligase Assay Kit (Catalog No. BPL100K)** contains the reagents for 100 assays in a 384-well plate assay format including 400 µl of 10 x Buffer, 35 µl of 100 x Biotin, 35 µl of 100 x hydroxylamine, 35 µl of 100 x ATP, 35 µl of 100 x pyrophosphatase (PPase, 10 U/ml) and 5 ml of Dye MPA3000 for phosphate detection. *E. coli* BirA is not included.

The ***E. coli* Biotin Protein Ligase Assay Kit Plus-100 (Catalog No. BPL100KE)** contains the reagents for 100 assays in a 384-well plate assay format including 400 µl of 10 x Buffer, 35 µl of 100 x Biotin, 35 µl of 100 x hydroxylamine, 35 µl of 100 x ATP, 35 µl of 100 x *E. coli* BirA (5000 nM), 35 µl of 100 x pyrophosphatase (PPase, 10 U/ml) and 5 ml of Dye MPA3000 for phosphate detection.

ASSAY PROTOCOL

The following assay protocol is based on the 384-well plate assay format. The reaction volume is 30 µl

ProFoldin
10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Phone: (508) 735-2539 **FAX: (508) 845-9258**
www.profoldin.com info@profoldin.com

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 H_2O, 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 3.3µl of 100 x Biotin, 3.3 µl of 100 x hydroxylamine, 3.5 µl of 100 x ATP with 23.1µl 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 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

ProFoldin offers high throughput assays for various drug targets and enzymes including DNA replication enzymes, RNA polymerases, kinases, bacterial cell wall synthesis enzymes and various metabolism enzymes, etc. For more information, please visit www.profoldin.com or send requests to info@profoldin.com.