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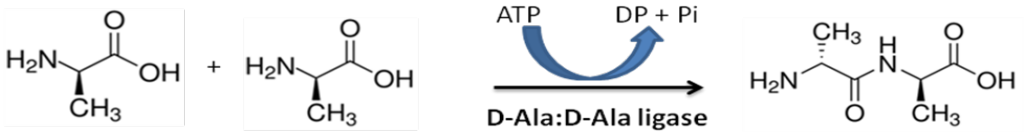
INSTRUCTIONS

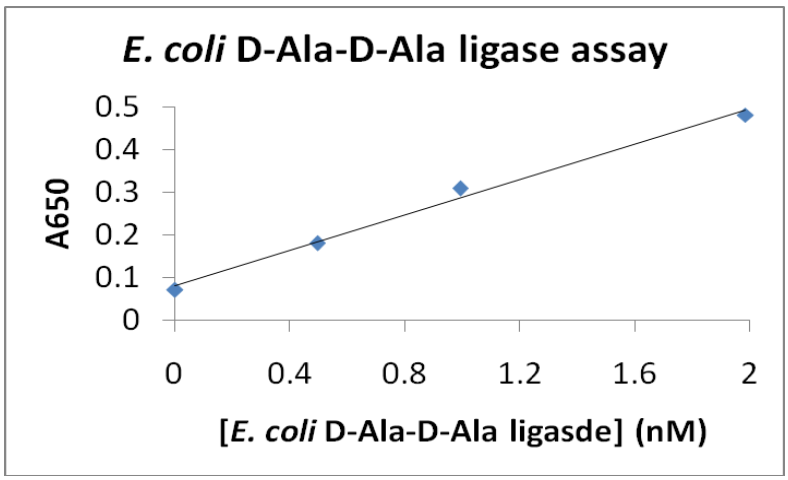
ProFoldin
E. coli D-Alanine :D-Alanine Ligase Assay Kits

D-Alanine: D-Alanine Ligase Assay Kit **Catalog No. DDA100K**
***E. coli* D-Alanine: D-Alanine Ligase Assay Kit Plus-100** **Catalog No. DDA100KE**

INTRODUCTION

D-Alanine-D-Alanine is one of the building blocks in peptidoglycan biosynthesis in bacteria. This dipeptide is generated by ligation between two D-Alanine molecules catalyzed by D-Alanine : D-Alanine ligase. The ligation reaction is coupled to the hydrolysis of ATP forming ADP and inorganic phosphate. The ***E. coli* D-Alanine :D-Alanine Ligase Assay** is based on measurement of the inorganic phosphate generated from the D-Alanine : D-Alanine ligation reaction. The inorganic 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. The high throughput assay can be used for screening inhibitors of *E.coli* D-Alanine : D-Alanine ligase in drug discovery research.





The **D-Alanine: D-Alanine Ligase Assay Kit (Catalog No. DDA100K)** 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 Enzyme Substrate and 5 ml of Dye MPA3000 for phosphate detection. *E. coli* D-Ala:D-Ala ligase is not included.

The ***E. coli* D-Alanine: D-Alanine Ligase Assay Kit Plus-100 (Catalog No. DDA100KE)** contains the reagents for100 assays in a 384-well plate assay format including 400 µl of 10 x Buffer, 35 µl of 100 x Enzyme Substrate, 35 µl of 100 x *E. coli* D-Ala:D-Ala ligase (200 nM) and 5 ml of Dye MPA3000 for phosphate detection.

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ASSAY PROTOCOL

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 H_2O, 33 µl of 10 x Buffer and 3.3 µl of 100 x *E. coli* D-Ala:D-Ala ligase.

(2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3µl of 100 x Enzyme substrate with 29.7µl 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

E. coli D-alanine D-alanine ligase Assay Kit Plus-500	DDA500KE
E. coli MurA Assay Kit Plus-100	MURA100KE
E. coli MurC Assay Kit Plus-100	MURC100KE
E. coli MurD Assay Kit Plus-100	MURD100KE
E. coli MurE Assay Kit Plus-100	MURE100KE
E. coli MurF Assay Kit Plus-100	MURF100KE
E. coli GlmU Assay Kit Plus-100	GLU100KE

More information of drug targets and enzyme assays

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.

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