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INSTRUCTIONS

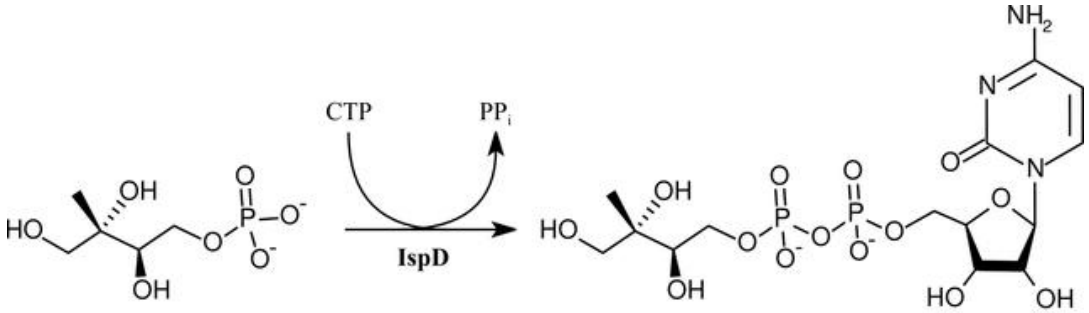
ProFoldin
E. coli Methylerythritol Phosphate Cytidyltransferase (IspD) Assay Kits

***E. coli* Methylerythritol Phosphate Cytidyltransferase (IspD) Assay Kit Plus-100**
Cat # ISPD100KE

***E. coli* Methylerythritol Phosphate Cytidyltransferase (IspD) Assay Kit Plus-500**
Cat # ISPD500KE

INTRODUCTION

Methylerythritol phosphate cytidyltransferase (IspD) is one of the enzymes in the non-mevalonate pathway for isoprenoid biosynthesis that present in many pathogenic organisms and plants but absent in mammals. IspD is an attractive target for the development of novel antibiotics and herbicides. This enzyme catalyzes the formation of 4-diphosphocytidyl-2-C-methyl-D-erythritol from CTP and 2-C-methyl-D-erythritol 4-phosphate (MEP).



2-*C*-Methylerythritol 4-phosphate (MEP)

4-Diphosphocytidyl-2-*C*-methyl-D-erythritol (CDP-ME)

The ***E. coli* Methylerythritol Phosphate Cytidyltransferase (IspD) Assay** is based on measurement of the pyrophosphate generated from the IspD reaction. The pyrophosphate is converted to phosphate by pyrophosphatase and the phosphate is detected by light absorbance at 650 nm. The assay is in a 384-well or 96-well plate format. It can be used for high throughput screening in drug discovery.

The ***E. coli* Methylerythritol Phosphate Cytidyltransferase (IspD) Assay Kit Plus-100 (Catalog # ISPD100KE)** 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 MEP, 35 µl of 100 x CTP, 35 µl of 100 x *E. coli* IspD (200 nM), 35 µl of 100 x pyrophosphatase (PPase, 10 U/ml), and 5 ml of Dye MPA3000 for phosphate detection.

The ***E. coli* Methylerythritol Phosphate Cytidyltransferase (IspD) Assay Kit Plus-500 (Catalog # ISPD100KE)** contains the reagents for 500 assays in a 384-well plate assay format including 2000 µl of

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10 x Buffer, 170 µl of 100 x MEP, 170 µl of 100 x CTP, 170 µl of 100 x *E. coli* IspD (200 nM), 170 µl of 100 x pyrophosphatase (PPase, 10 U/ml), and 25 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 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 257.4 µl of H_2O, 33 µl of 10 x Buffer, 3.3 µl of 100 x *E. coli* IspD and 3.3 µl of 100 x PPase .

(2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3µl of 100 x MEP, 3.3µl of 100 x CTP with 26.4µl 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.