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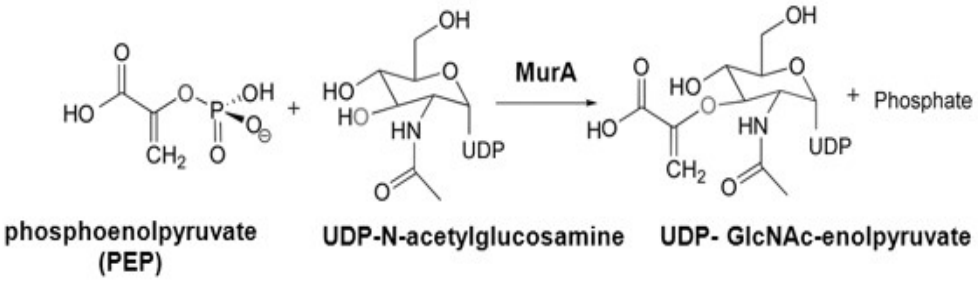
INSTRUCTIONS

ProFoldin
MurA Assay Kits

***E. coli* MurA Assay Kit Plus-100** **Catalog No. MURA100KE**
***E. coli* MurA Assay Kit Plus-500** **Catalog No. MURA500KE**

INTRODUCTION

MurA or UDP-N-acetylglucosamine enolpyruvyl transferase catalyzes the first committed step in peptidoglycan biosynthesis in bacteria. It is an essential enzyme and attractive target for anti-bacterial drug discovery. MurA transfers enolpyruvate from phosphoenolpyruvate (PEP) to uridine diphospho-*N*-acetylglucosamine (UNAG) generating enolpyruvyl-UDPN-acetylglucosamine (EP-UNAG) and inorganic phosphate.



The ***E. coli* MurA Assay** is based on measurement of the inorganic phosphate generated from the MurA 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. 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* MurA in drug discovery research. It may also be used for characterization of *E.coli* MurA.

The ***E. coli* MurA Assay Kit Plus-100 (Catalog No. MURA100KE)** contains the reagents for100 assays in a 384-well plate assay format including 400 µl of 10 x Assay buffer, 35 µl of 100 x PEP, 35 µl of 100 x UDP-N-acetylglucosamine (UGN), 35 µl of 100 x *E. coli* MurA (5000 nM) and 5 ml of Dye MPA3000 for phosphate detection.

The ***E. coli* MurA Assay Kit Plus-500 (Catalog No. MURA500KE)** contains the reagents for 500 assays in a 384-well plate assay format including 2000 µl of 10 x Assay buffer, 170 µl of 100 x PEP, 170 µl of 100 x UDP-N-acetylglucosamine (UGN), 170 µl of 100 x *E. coli* MurA (5000 nM) 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

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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* MurA.

(2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3 µl of 100 x PEP and 3.3 µl of 100 x UGN and 26.4 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 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 LINEARITY TEST

Follow the same protocol described above except mixing 27 µl of the premix with 3 µl of the 10 x Enzyme substrate at different time points. Plot the reaction signal versus the reaction time to define the linear range.

IC50 MEASUREMENT OF ENZYME INHIBITORS

The concentration range of the inhibitor to be tested depends on the potency of the inhibitor. In general, the maximum concentration is about 10 to 20 fold higher than the IC50 value. The following protocol is for IC50 measurement of one inhibitor with IC50 values around 10 µM.

1. In 8 assay wells, add 0.6 µl of 2-fold serial dilution solutions of the inhibitor from 5 mM to 0.039 mM in water or DMSO. In one control well, add 0.6 µl of water or DMSO. In another control well, add 0.6 µl of 1 M EDTA.
2. Prepare 297 µl of premix and 33 µl of 10 x Enzyme substrate as described above.
3. Mix 26.4 µl of the premix and 0.6 µl of the 50 x inhibitor for 5 min.
4. Add 3 µl of the 10 x Enzyme substrate and incubate the assay reaction for the time in the linear range.
5. 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.
6. Calculate IC50s using a computer IC50 fitting software.

Note: If DMSO is used to make the inhibitor solutions, the final concentration of DMSO in the assay is 2 %. It is important to make sure that 2 % DMSO does not affect the enzyme activity. Otherwise, the assay condition should be adjusted accordingly to keep the sufficient signal to background ratio and the assay linearity.