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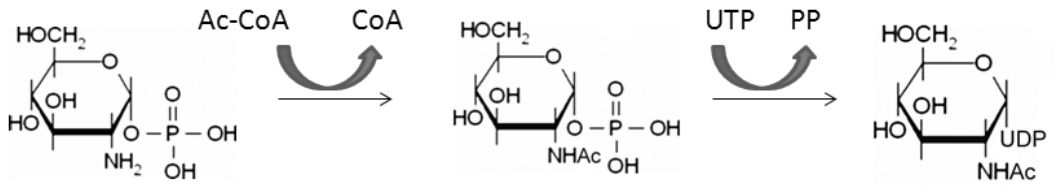
INSTRUCTIONS

ProFoldin
E. coli UDP-N-acetylglucosamine pyrophosphorylase (GlmU) Assay Kits

E. coli **GlmU Assay Kit Plus-100** **Cat # GLU100KE**
E. coli **GlmU Assay Kit Plus-500** **Cat # GLU500KE**

INTRODUCTION

UDP-N-acetylglucosamine pyrophosphorylase (GlmU) is a bifunctional enzyme that catalyzes transfer of acetyl and uridyl groups onto glucosamine-1-P to generate UDP-GlcNAc, an essential precursor of peptidoglycans. UDP-GlcNAc is involved in the synthesis of the N-acetylglucosamine polysaccharide adhesin required for biofilm formation in bacteria.



Glucosamine-1-P **N-Acetyl-glucosamine-1-P** **UDP-Glc-NAc**

The ***E. coli*** **UDP-N-acetylglucosamine pyrophosphorylase (GlmU) Assay** is based on measurement of the pyrophosphate generated from the GlmU reaction. 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. The high throughput assay can be used for screening inhibitors of *E.coli* GlmU in drug discovery research. It may also be used for characterization of *E.coli* GlmU.

The ***E. coli*** **GlmU Assay Kit Plus-100 (Catalog No. GLU100KE)** contains the reagents for 100 assays in a 384-well plate assay format including 400 µl of 10 x Buffer, 33 µl of 100 x Enzyme Substrate composed of 2.5 mM glucosamine-1-P, 2.5 mM acetyl CoA and 2.5 mM UTP, 33 µl of 100 x *E. coli* GlmU (500 nM), 33 µl of 100 x pyrophosphatase (PPase, 10 U/ml), and 5 ml of Dye MPA3000 for phosphate detection.

The ***E. coli*** **GlmU Assay Kit Plus-500 (Catalog No. GLU500KE)** contains the reagents for 500 assays in a 384-well plate assay format including 2000 µl of 10 x Buffer, 170 µl of 100 x Enzyme Substrate, 170 µl of 100 x *E. coli* GlmU (500 nM), 170 µl of 100 x pyrophosphatase (PPase, 10 U/ml), and 25 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 257.4 µl of H_2O, 33 µl of 10 x Buffer, 3.3 µl of 100 x *E. coli* GlmU and 3.3 µl of 100 x PPase .

(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 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.