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INSTRUCTIONS

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S. pneumoniae MurC Assay Kits

S. pneumoniae MurC Assay Kit Plus-100

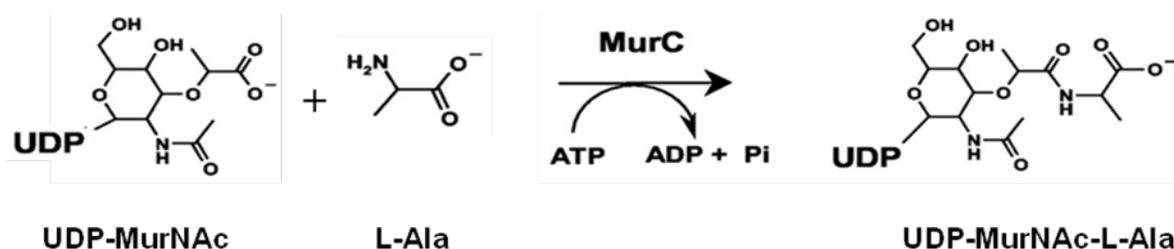
Catalog No. MURC100KN

S. pneumoniae MurC Assay Kit Plus-500

Catalog No. MURC500KN

INTRODUCTION

MurC or UDP-N-acetylmuramic acid:L-alanine ligase is the first of four paralogous amino acid-adding enzymes in the pathway of peptidoglycan biosynthesis in bacteria. It is an essential enzyme and attractive target for anti-bacterial drug discovery. MurC catalyzes the addition of L-alanine onto the nucleotide precursor UDP-MurNAc generating UDP-MurNAc-L-Ala. The ligation reaction is coupled to the hydrolysis of ATP forming ADP and inorganic phosphate.



The *S. pneumoniae* MurC Assay is based on measurement of the inorganic phosphate generated from the MurC 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 if a plate reader is not available. The high throughput assay can be used for screening inhibitors of *E. coli* MurC in drug discovery research. It may also be used for characterization of *E. coli* MurC.

The *S. pneumoniae* MurC Assay Kit Plus-100 (Catalog No. MURC100KE) contains the reagents for 100 assays in a 384-well plate assay format including 400 μl of 10 x Assay buffer, 35 μl of 100 x UDP-MurNAc (4 mM), 35 μl of 100 x L-Ala (4 mM), 35 μl of 100 x ATP (10 mM), 35 μl of 100 x *S. pneumoniae* MurC (5000 nM) and 5 ml of Dye MPA3000 for phosphate detection.

The *S. pneumoniae* MurC Assay Kit Plus-500 (Catalog No. MURC500KE) 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 UDP-MurNAc (4 mM), 170 μl of 100 x L-Ala (4 mM), 170 μl of 100 x ATP (10 mM), 170 μl of 100 x *S. pneumoniae* MurC (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 μ l. For 96-well plate assays, the reaction volume is 60 μ l and the final assay volume is 150 μ l. For detection using a cuvette, the reaction volume is 400 μ l and the final assay volume is 1000 μ l.

1. Reagent preparation:

For each 10 assay reactions,

- (1) Prepare 297 μ l of premix composed of 261 μ l of H₂O, 33 μ l of 10 x Buffer and 3.3 μ l of 100 x *S. pneumoniae* MurC.
- (2) Prepare 33 μ l of 10 x Enzyme substrate by mixing 3.3 μ l of 100 x UDP-MurNAc, 3.3 μ l of 100 x L-Ala, 3.3 μ l of 100 x ATP, and 23.1 μ l of water.

2. Reaction:

Mix 27 μ l of the premix with 3 μ l of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at 37°C for 60 min.

3. Detection:

Add 45 μ l of the Dye MPA3000 into the 30 μ l 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

<i>E. coli</i> MurC assay Kit Plus-100	MURC-100KE
<i>P. aeruginosa</i> MurC assay Kit Plus-100	MURC-100KP
<i>E. coli</i> MurA Assay Kit Plus-100	MURA100KE
<i>E. coli</i> MurD Assay Kit Plus-100	MURD100KE
<i>P. aeruginosa</i> MurD Assay Kit Plus-100	MURD100KP
<i>S. aureus</i> MurD Assay Kit Plus-100	MURD100KS
<i>E. coli</i> MurE Assay Kit Plus-100	MURE100KE
<i>E. coli</i> MurF Assay Kit Plus-100	MURF100KE
<i>S. pneumoniae</i> MurF Assay Kit Plus-100	MURF100KN
<i>P. aeruginosa</i> MurF Assay Kit Plus-100	MURF100KP
<i>S. aureus</i> MurF Assay Kit Plus-100	MURF100KS
<i>E. coli</i> D-Alanine: D-Alanine Ligase Assay Kit Plus-100	DDA100KE
<i>E. coli</i> GlmU Assay Kit Plus-100	GLU100KE

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