



## ProFoldin

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# INSTRUCTIONS

## ProFoldin

### *E. coli* MurD Assay Kits

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*E. coli* MurD Assay Kit Plus-100

Catalog No. MURD100KE

*E. coli* MurD Assay Kit Plus-500

Catalog No. MURD500KE

#### INTRODUCTION

MurD is a D-Glutamic acid-adding enzyme in the pathway for bacterial cell-wall peptidoglycan synthesis. It is an essential enzyme and attractive target for anti-bacterial drug discovery. MurD catalyses the addition of D-glutamic acid to UDP-MurNAc-L-Ala, generating UDP-MurNAc-dipeptide. The ligation reaction uses ATP hydrolysis as an energy source forming ADP and inorganic phosphate.



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

The *E. coli* MurD Assay Kit Plus-100 (Catalog No. MURD100KE) contains the reagents for 100 assays in a 384-well plate assay format including 400  $\mu\text{l}$  of 10 x Buffer, 35  $\mu\text{l}$  of 100 x UDP-MurNAc-L-Ala (UMA), 35  $\mu\text{l}$  of 100 x D-Glu, 35  $\mu\text{l}$  of 100 x ATP, 35  $\mu\text{l}$  of 100 x *E. coli* MurD (2000 nM) and 5 ml of Dye MPA3000 for phosphate detection.

The *E. coli* MurD Assay Kit Plus-500 (Catalog No. MURD500KE) contains the reagents for 500 assays in a 384-well plate assay format including 2000  $\mu\text{l}$  of 10 x Buffer, 170  $\mu\text{l}$  of 100 x UDP-MurNAc-L-Ala (UMA), 170  $\mu\text{l}$  of 100 x D-Glu, 35  $\mu\text{l}$  of 100 x ATP, 170  $\mu\text{l}$  of 100 x *E. coli* MurD (2000 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  $\mu\text{l}$

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and the final assay volume is 75  $\mu$ l. For 96-well plate assays, the reaction volume is 60  $\mu$ l and the final assay volume is 150  $\mu$ l. For detection using a cuvette, the reaction volume is 400  $\mu$ l and the final assay volume is 1000  $\mu$ l.

### 1. Reagent preparation:

For each 10 assay reactions,

- (1) Prepare 297  $\mu$ l of premix composed of 261  $\mu$ l of H<sub>2</sub>O, 33  $\mu$ l of 10 x Buffer and 3.3  $\mu$ l of 100 x *E. coli* MurD.
- (2) Prepare 33  $\mu$ l of 10 x Enzyme substrate by mixing 3.3  $\mu$ l of 100 x UMA, 3.3  $\mu$ l of 100 x D-Glu, 3.3  $\mu$ l of 100 x ATP, and 23.1  $\mu$ l of water.

### 2. Reaction:

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

### 3. Detection:

Add 45  $\mu$ l of the Dye MPA3000 into the 30  $\mu$ 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

E. coli MurA Assay Kit Plus-100	MURA100KE
E. coli MurC Assay Kit Plus-100	MURC100KE
S. pneumoniae MurC Assay Kit Plus-100	MURC100KN
P. aeruginosa MurD Assay Kit Plus-100	MURD100KP
S. aureus MurD Assay Kit Plus-100	MURD100KS
E. coli MurF Assay Kit Plus-100	MURF100KE
P. aeruginosa MurF Assay Kit Plus-100	MURF100KP
S. aureus MurF Assay Kit Plus-100	MURF100KS
S. pneumoniae MurF Assay Kit Plus-100	MURF100KN
E. coli MurE Assay Kit Plus-100	MURE100KE
E. coli GlnU Assay Kit Plus-100	GLU100KE
E. coli D-alanine ligase Assay Kit Plus-100	DDA100KE

## More information of drug targets and enzyme assays

For more information of drug targets and enzyme assays, please visit [www.profoldin.com](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).