

**ProFoldin**

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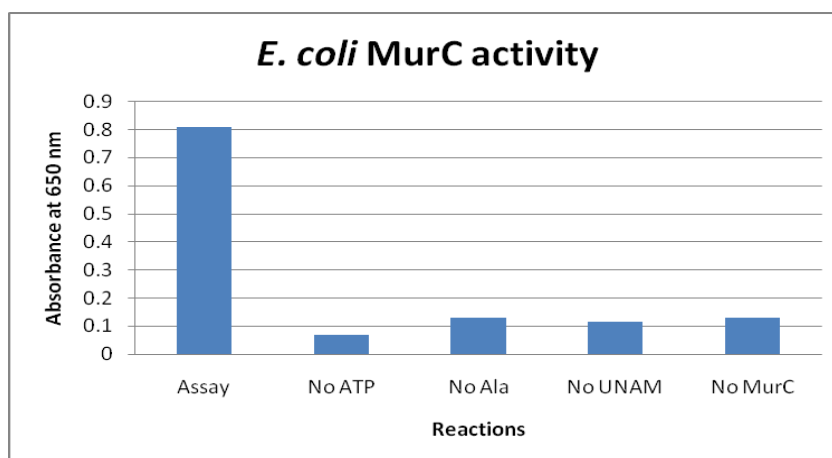
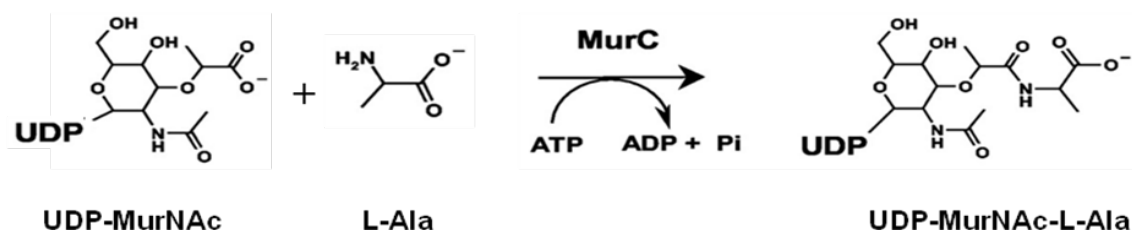
INSTRUCTIONS

ProFoldin *E. coli* MurC

E. coli MurC – for 100 assays

Catalog No. MURC-100EC

Protein construct:	Wild-type <i>E. coli</i> MurC or UDP-N-acetylmuramic acid : L-alanine ligase purified from a bacterial expression system.
MW:	54 kDa
Enzyme concentration:	5 μ M MurC with 8 μ M UDP-MurNAc for stabilization.
Enzyme activity assay:	The MurC activity is measured by using the <i>E. coli</i> MurC Assay Kit Plus-100 (Catalog No. MURC100KE)
Storage temperature:	-20 or -80°C. Do not freeze-and-thaw repeatedly.
Enzyme dilution:	Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution



The *E. coli* MurC – for 100 assays (Catalog No. MURC-100EC) includes 33 μ l of 100 x *E. coli* MurC (5 μ M).

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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 261 µl of H₂O, 33 µl of 10 x Buffer and 3.3 µl of 100 x *E. coli* MurC.
- (2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3 µl of 100 x UDP-MurNAc, 3.3 µl of 100 x L-Ala, 3.3 µl of 100 x ATP, and 23.1 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 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	MURC100KE
<i>S. pneumoniae</i> MurC assay Kit Plus-100	MURC-100KN
<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>E. coli</i> MurE Assay Kit Plus-100	MURE100KE
<i>E. coli</i> MurF Assay Kit Plus-100	MURF100KE

More information of drug targets and enzyme assays

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