ProFoldin
10 Technology Drive, Suite 40
Hudson, MA 01749-2791 USA
Tel: (508) 735-2539 FAX: (508) 845-9258
www.profoldin.com
info@profoldin.com

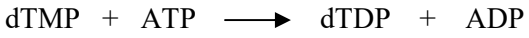
INSTRUCTIONS

ProFoldin
E. coli Thymidylate Kinase Assay Kit Plus

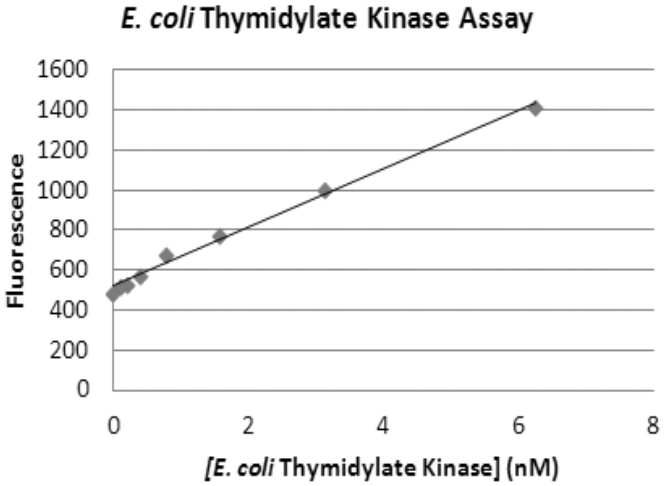
***E. coli* Thymidylate Kinase Assay Kit Plus** **Catalog number: TMK100KE**
***E. coli* Thymidylate Kinase Assay Kit Plus-500** **Catalog number: TMK500KE**

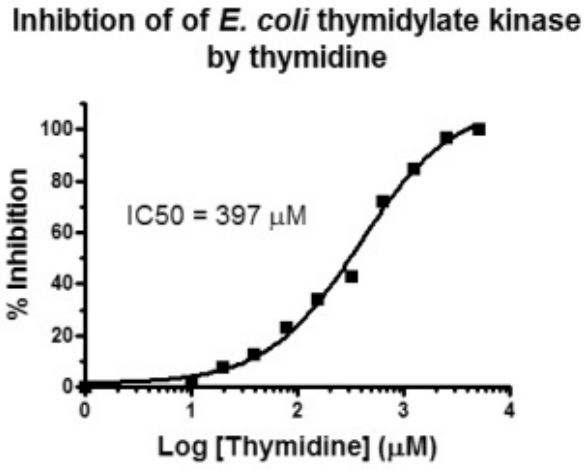
Introduction

Thymidylate kinase is an important enzyme in the dTTP synthesis pathway for DNA synthesis. The function of this enzyme is to catalyze the phosphorylation of dTMP using ATP to form dTDP and ADP.



Thymidylate kinase is an attractive antibacterial target. The thymidylate kinase assay is based on measurement of ADP generated from the kinase reaction. The assay is fluorescence-based and can be carried out using regular black 96-well or 384-well plates or micro-cuvettes.





The ***E. coli* Thymidylate Kinase Assay Kit Plus** (Catalog No. TMK100KE) includes 600 µl of 10 x Buffer, 35 µl of 100 x dTMP, 35 µl of 100 x *E. coli* Thymidylate kinase, 35 µl of 100 x ATP, 35 µl of 100 x MUK Reagent A, 35 µl of 100 x MUK Reagent B and 300 µl of 10 x fluorescence dye for 100 assays in a 384-well assay format.

The ***E. coli* Thymidylate Kinase Assay Kit Plus-500** (Catalog No. TMK500KE) includes 2000 µl of 10 x Buffer, 170 µl of 100 x dTMP, 170 µl of 100 x *E. coli* Thymidylate kinase, 170 µl of 100 x ATP, 170 µl of 100 x MUK Reagent A, 170 µl of 100 x MUK Reagent B and 1700 µl of 10 x fluorescence dye for 500 assays in a 384-well assay format.

ProFoldin
10 Technology Drive, Suite 40
Hudson, MA 01749-2791 USA
Tel: (508) 735-2539 **FAX: (508) 845-9258**
www.profoldin.com
info@profoldin.com

INSTRUCTIONS

Assay Protocol

1. **Reagent preparation:**
   (1) 1 x reaction buffer: dilute the 10 x Buffer 10-fold with water. The 1 x buffer is composed of 50 mM Tris-HCl, pH 8.0, 3 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 0.003% Brij-35.
   (2) 10 x dTMP: dilute the 100 x dTMP solutions (5 mM) 10-fold with water.
   (3) 10 x ATP: dilute the 100 x ATP solutions (5 mM) 10-fold with water.
   (4) 10 x thymidylate kinase: dilute the 100 x thymidylate kinase 10-fold with 1 x buffer.
   (5) 10 x MUK Reagent A: dilute the 100 x MUK Reagent A 10-fold with water.
   (6) 10 x MUK Reagent B: dilute the 100 x MUK Reagent B 10-fold with 1 x buffer.
   (7) 1 x fluorescence dye: dilute the 10 x Fluorescence dye 10-fold with water.

2. **Kinase assay**
   The kinase reaction volume is 30 µl.
   (1) In a standard black 384-well plate (Matrix 4318), mix 12 µl of water, 3 µl of 10 x Buffer, 3 µl of 10 x dTMP, 3 µl of 10 x ATP, 3 µl of 10 x kinase. Incubate the reaction for 2 min.
   (2) Add 3 µl of 10 x MUK Reagent A, 3 µl of 10 x MUK Reagent B.
   (3) Incubate the reaction mixture at 37°C for 60 min.
   (4) Add 30 µl of the 1 x fluorescence dye into the 30 µl of the reaction mixture.
   (5) Measure the fluorescence intensity at 535 nm with excitation at 485 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.

Reference:

Choi J.Y. et al, Structure Guided Development of Novel Thymidine Mimetics targeting Pseudomonas aeruginosa Thymidylate Kinase: from Hit to Lead Generation, J Med Chem. January 26; 55(2): 852–870 (2012).

Related products

E. coli Guanylate Kinase Assay Kit Plus-500	Catalog number: GMK500KE
E. coli NAD+ Kinase Assay Kit Plus-500	Catalog number: NAK500KE
E. coli UMP Kinase Assay Kit Plus-500	Catalog number: UMK500KE
Human Thymidylate Kinase Assay Kit Plus-500	Catalog number: HTMK500KE

For more information of drug targets and enzyme assays, please visit www.profoldin.com.