



## ProFoldin

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## INSTRUCTIONS

# ProFoldin Human Thymidylate Kinase Assay Kit Plus-500

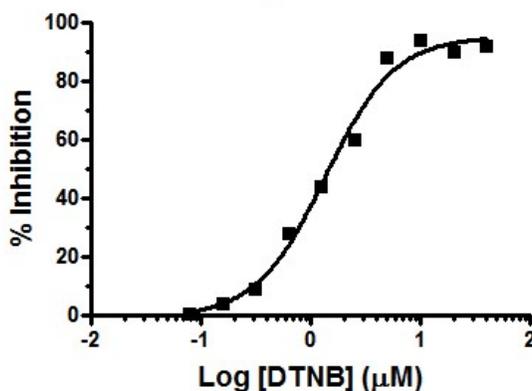
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Catalog No: HTMK500KE

### Introduction

Thymidylate kinase is an important enzyme in the dTTP synthesis pathway for DNA synthesis. The thymidylate kinase activity is higher in the tumor than in the corresponding normal human tissue. The function of this enzyme is to catalyze the phosphorylation of dTMP using ATP to form dTDP and ADP. 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 or white 96-well or 384-well plates or micro-cuvettes.

**Inhibition of human thymidylate kinase  
by DTNB**



The **Human Thymidylate Kinase Assay Kit Plus-500** (Catalog No. HTMK500KE) includes 1700  $\mu$ l of 10 x reaction buffer, 20  $\mu$ l of 1000 x human thymidylate kinase (50  $\mu$ M), 170  $\mu$ l of 100 x ATP (5 mM), 170  $\mu$ l of 100 x dTMP (40 mM), 170  $\mu$ l of 100 x MUK Reagent A, 170  $\mu$ l of 100 x MUK Reagent B and 1700  $\mu$ l of 10 x fluorescence dye. The kit reagents are sufficient for 500 thymidylate kinase assays in a 384-well plate assay format or 300 thymidylate kinase assays in a 96-well plate assay format.

### Assay protocol

The following assay protocol is based on 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 50  $\mu$ l and the final assay volume is 100  $\mu$ l. For 384-well plate assays (plate type: Matrix 4318 or alike). The reaction volume is 30  $\mu$ l and the final assay volume is 60  $\mu$ l.

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## 1. Reagent preparation:

For each 10 assay reactions,

- (1) Prepare 55  $\mu$ l of 10 x ATP by dilution of 5.5  $\mu$ l of 100 x ATP with 49.5  $\mu$ l of water.
- (2) Prepare 550  $\mu$ l of 1 x fluorescence dye by dilution of 55  $\mu$ l of 10 x Fluorescence dye with 495  $\mu$ l of water.
- (3) Prepare 385  $\mu$ l of premix composed of 324  $\mu$ l of H<sub>2</sub>O, 55  $\mu$ l of 10 x Buffer, 5.5  $\mu$ l of 100 x dTMP and 0.5  $\mu$ l of 1000 x thymidylate kinase.

## 2. Kinase assay

In each well,

- (1) Mix 35  $\mu$ l of the premix with 5  $\mu$ l of 10 x ATP. Incubate the reaction at 37°C for 2 min.
- (2) Add 5  $\mu$ l of 10 x MUK Reagent A and 5  $\mu$ l of 10 x MUK Reagent B.
- (3) Incubate the reaction mixture at 37°C for 60 min.
- (4) Add 50  $\mu$ l of the 1 x fluorescence dye into the 50  $\mu$ l of the reaction mixture.
- (5) Measure the fluorescence intensity at 535 nm with excitation at 485 nm.

Note: The 1 x reaction buffer is 50 mM Tris-HCl, pH 8.0, 3 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 50 mM NaCl, 0.003% Brij-35. Diluted thymidylate kinase is freshly prepared before the assay.

## 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

MicroMolar Universal Kinase Assay Kit-500  
*E. coli* Thymidylate Kinase Assay Kit Plus-500  
*E. coli* Guanylate Kinase Assay Kit Plus-500  
*E. coli* UMP Kinase Assay Kit Plus-500  
*E. coli* NAD<sup>+</sup> Kinase Assay Kit Plus-500

Catalog number: MUK500K  
Catalog number: TMK500KE  
Catalog number: GMK500KE  
Catalog number: UMK500KE  
Catalog number: NAK500KE

## 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). For HTS quantities of assay kit components, please send request to [info@profoldin.com](mailto:info@profoldin.com).