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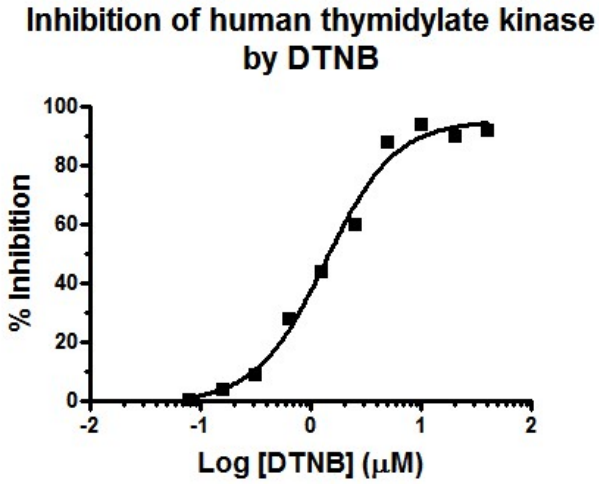
INSTRUCTIONS

ProFoldin
Human Thymidylate Kinase

Human Thymidylate Kinase – for 500 assays Catalog No: HTMK-500

Protein construct:	Wild-type Human Thymidylate Kinase purified from a bacterial protein expression system.
MW:	24 kDa
Enzyme concentration:	50 µM
Enzyme assay:	The kinase activity of human thymidylate kinase is measured by using the Human Thymidylate Kinase Assay kit (Catalog No. HTMK500K).
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 **Human Thymidylate Kinase – for 500 assays (Catalog No: HTMK-500)** includes 20 µl of 1000 x human thymidylate kinase (50 µM). It is for 500 assays.

Assay Protocol using the Human Thymidylate Kinase Assay kit

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

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1. Reagent preparation:

For each 10 assay reactions,

(1) Prepare 55 µl of 10 x ATP by dilution of 5.5 ul of 100 x ATP with 49.5 µl of water.

(2) Prepare 550 µl of 1 x fluorescence dye by dilution of 55 µl of 10 x Fluorescence dye with 495 µl of water.

(3) Prepare 385 µl of premix composed of 324 µl of H_2O, 55 µl of 10 x Buffer, 5.5 µl of100 x dTMP and 0.5 µl of 1000 x thymidylate kinase.

2. Kinase assay

In each well,

(1) Mix 35 µl of the premix with 5 µl of 10 x ATP. Incubate the reaction at 37°C for 2 min.

(2) Add 5 µl of 10 x MUK Reagent A and 5 µl of 10 x MUK Reagent B.

(3) Incubate the reaction mixture at 37°C for 60 min.

(4) Add 50 µl of the 1 x fluorescence dye into the 50 µl 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 $MgCl_2$, 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	Catalog number: MUK500K
E. coli Thymidylate Kinase Assay Kit Plus-500	Catalog number: TMK500KE
E. coli Guanylate Kinase Assay Kit Plus-500	Catalog number: GMK500KE
E. coli UMP Kinase Assay Kit Plus-500	Catalog number: UMK500KE
E. coli NAD^+ Kinase Assay Kit Plus-500	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.