



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* UMP Kinase Assay Kit Plus

E. coli UMP Kinase Assay Kit Plus-100

Catalog number: **UMK100KE**

E. coli UMP Kinase Assay Kit Plus-500

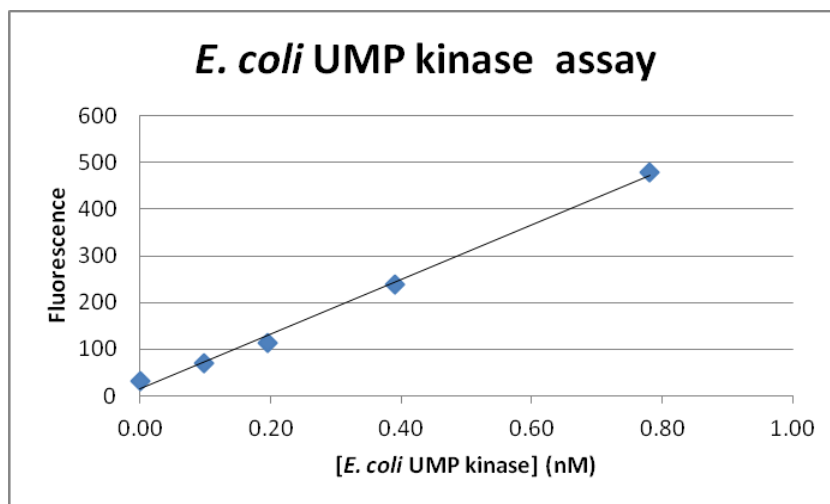
Catalog number: **UMK500KE**

Introduction

UMP kinase or uridylylate kinase is essential for biosynthesis of nucleic acid. It catalyzes the ATP-dependent phosphorylation of UMP into UDP.



UMP kinase is an attractive antibacterial target. The UMP 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* UMP Kinase Assay Kit Plus (Catalog No. **UMK100KE**) includes 600 μ l of 10 x assay buffer, 35 μ l of 100 x UMP, 35 μ l of 100 x *E. coli* UMP kinase, 35 μ l of 100 x ATP, 35 μ l of 100 x MUK Reagent A, 35 μ l of 100 x MUK Reagent B and 300 μ l of 10 x fluorescence dye for 100 assays in a 384-well assay format.

The *E. coli* UMP Kinase Assay Kit Plus-500 (Catalog No. **UMK500KE**) includes 2 ml of 10 x assay buffer, 170 μ l of 100 x UMP, 170 μ l of 100 x *E. coli* UMP kinase, 170 μ l of 100 x ATP, 170 μ l of 100 x MUK Reagent A, 170 μ l of 100 x MUK Reagent B and 1.7 ml of 10 x fluorescence dye for 100 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 MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 0.003% Brij-35.
- (2) 10 x UMP: dilute the 100 x UMP solutions (10 mM) 10-fold with water.
- (3) 10 x ATP: dilute the 100 x ATP solutions (2 mM) 10-fold with water.
- (4) 10 x UMP kinase: dilute the 100 x UMP 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 μ l.

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

Assay Protocol for enzyme inhibition

In order to accurately measure the IC₅₀, it is important to make sure the assay is in the linear range. The linear range can be defined by enzyme concentration-dependence and time-dependence experiments. The enzyme concentration and time in the linear range is selected for the IC₅₀ measurement.

For the enzyme inhibition experiment, typically 50 x stock solutions with a 2-fold serial dilution in water or DMSO are prepared. In the wells of the 384-well plate, 0.6 μ l of the 50 x inhibitor is mixed with a premix composed of the buffer and enzyme for 5 min. Then the substrate is added. After incubation for 2 min, 3 μ l of 10 x MUK Reagent A and 3 μ l of 10 x MUK Reagent B are added. The total assay volume is 30 μ l. At the end of the reaction, the dye is added and the fluorescence intensity is measured.

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 Thymidylate Kinase Assay Kit Plus-500

Catalog number: TMK500KE

Human Thymidylate Kinase Assay Kit Plus-500

Catalog number: HTMK500KE

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