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
NAD^+ Kinase Assay Kit Plus

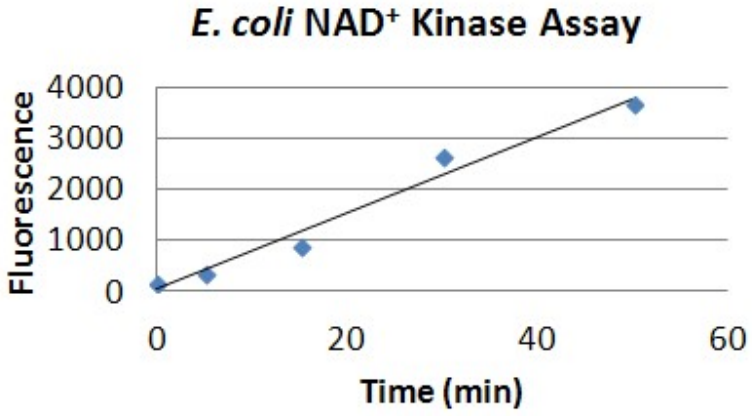
***E. coli* NAD^+ Kinase Assay Kit Plus-100** **Catalog number: NAK100KE**
***E. coli* NAD^+ Kinase Assay Kit Plus-500** **Catalog number: NAK500KE**

Introduction

NAD^+ kinase converts NAD^+ into $NADP^+$ by phosphorylation in the presence of ATP. $NADP^+$ plays key roles in energy transduction and various biochemical process including DNA repair, protein modification and cell signaling.

$$NAD^+ + ATP \longrightarrow NADP^+ + ADP$$

NAD^+ kinase is an attractive antibacterial target. The NAD^+ 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* NAD^+ Kinase Assay Kit Plus** (Catalog No. **NADK100KE**) includes 600 µl of 10 x assay buffer, 35 µl of 100 x NAD^+, 35 µl of 100 x *E. coli* NAD 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* NAD^+ Kinase Assay Kit Plus-500** (Catalog No. **NADK 500KE**) includes 2 ml of 10 x assay buffer, 170 µl of 100 x NAD^+,, 170 µl of 100 x *E. coli* NAD kinase, 170 µl of 100 x ATP, 170 µl of 100 x MUK Reagent A, 170 µl 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 Reaction Buffer 10-fold with water. The 1 x reaction 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 NAD^+: dilute the 100 x NAD^+ solutions (100 mM) 10-fold with water.
   (3) 10 x ATP: dilute the 100 x ATP solutions (2 mM) 10-fold with water.
   (4) 10 x NAD^+ kinase: dilute the 100 x NAD^+ kinase 10-fold with 1 x reaction 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 reaction 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 reaction buffer, 3 µl of 10 x NAD^+, 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

In order to accurately measure the IC_{50}, 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 IC_{50} 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 µl 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 µl of 10 x MUK Reagent A and 3 µl of 10 x MUK Reagent B are added. The total assay volume is 30 µl. 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 Thymidylate Kinase Assay Kit Plus-500	Catalog number: TMK500KE
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.