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INSTRUCTIONS

ProFoldin
MicroMolar Universal Kinase Assay Kits

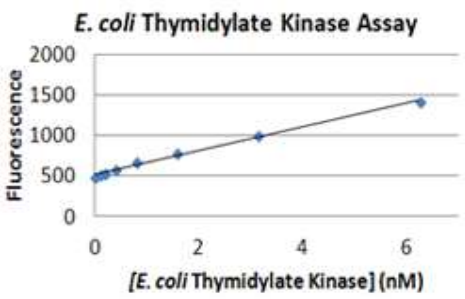
MicroMolar Universal Kinase Assay Kit **Catalog Number: MUK100K**
MicroMolar Universal Kinase Assay Kit-1000 **Catalog Number: MUK1000K**

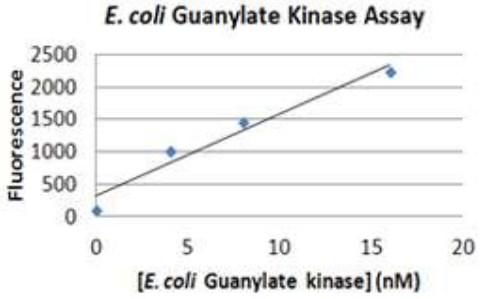
Introduction

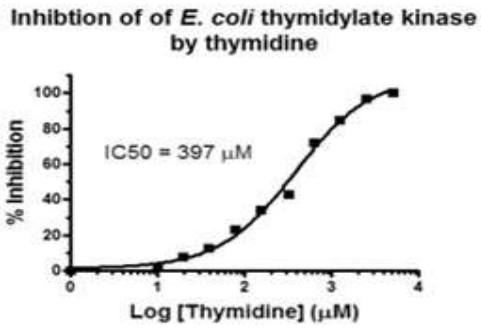
The MicroMolar Universal Kinase Assay Kit is based on measurement of ADP generated from the kinase reaction. It is used to measure the activities of purified kinases producing ADP at concentrations ranging from 0.1 μM to 10 μM.

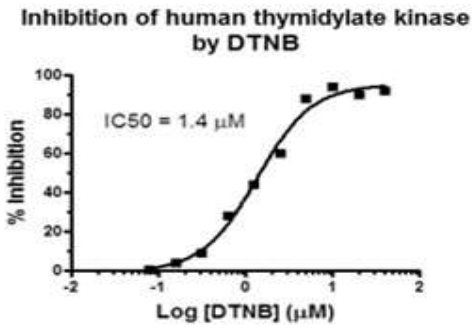
Substrate + ATP ⟶ Phosphorylated substrate + ADP

The assay is fluorescence-based and can be carried out using regular black or white 96-well or 384-well plates or micro-cuvettes. It is important to avoid using unnecessarily high ATP concentrations in the kinase assays for the following reasons. One is to allow the assay to detect ATP-competitive inhibitors. The other is to avoid a high ADP background from ADP contamination in the ATP samples. It is recommended to use ATP concentrations at 20 μM (micromolar).









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The **MicroMolar Universal Kinase Assay Kit** (Catalog No. **MUK100K**) includes 600 µl of 10 x assay buffer, 35 µl of 100 x ATP, 35 µl of 100 x MUK Reagent A, 35 µl of 100 x MUK Reagent B, 30 µl of 1 mM ADP and 350 µl of 10 x fluorescence dye for 100 assays in a 384-well assay format.

The **MicroMolar Universal Kinase Assay Kit-1000** (Catalog No. **MUK1000K**) includes 4000 µl of 10 x assay buffer, 350 µl of 100 x ATP, 350 µl of 100 x MUK Reagent A, 350 µl of 100 x MUK Reagent B, 200 µl of 1 mM ADP and 3500 µl of 10 x fluorescence dye for 1000 assays in a 384-well assay format.

Assay Protocol

1. **Reagent preparation:**
   (1) 1 x MUK Buffer: dilute the 10 x MUK Buffer 10-fold with water. The 1x MUK Buffer is composed of 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 3 mM $MgCl_2$, 0.5 mM DTT, 0.2 mM EDTA, 0.003% Brij-35.
   (2) 10 x MUK Reagent A: dilute the 100 x MUK Reagent A 10-fold with water
   (3) 10 x MUK Reagent B: dilute the 100 x MUK Reagent B 10-fold with 1x MUK Buffer
   (4) 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, mix 12 µl of water, 3 µl of 10 x reaction buffer, 3 µl of 10 x kinase substrate, 3 µl of 10 x ATP, 3 µl of freshly prepared 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.

Enzyme inhibition

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

Thymidylate Kinase Assay Kit Plus-500	Catalog number: TMK500KE
Guanylate Kinase Assay Kit Plus-500	Catalog number: GMK500KE
NAD+ Kinase Assay Kit Plus-500	Catalog number: NAK500KE
UMP Kinase Assay Kit Plus-500	Catalog number: UMK500KE
Human Thymidylate Kinase Assay Kit Plus-500	Catalog Number: HTMK500KE