

HIPK1 (Human), Active

Recombinant protein expressed in Sf9 cells

Cat# CY-SPH03

Lot No. P263-1
5 µg 0.1 µg/µl

Background:

HIPK1 or homeodomain-interacting protein kinase 1 is a ser/thr protein kinase and a member of the HIPK family. HIPK1 is a nuclear kinase that phosphorylates homeodomain transcription factors. HIPK1 phosphorylates DAXX and this leads to its relocalization and subsequent decrease in transcriptional repression activity (1). HIPK1 also interacts with p53 and phosphorylates it on serine residues. HIPK1 expression is elevated in breast cancer cell lines and embryonic fibroblasts from HIPK1-null mice show more susceptibility to apoptosis induced by DNA damage (2).

Product Description:

Recombinant human HIPK1 (156-555) was expressed by baculovirus in Sf9 cells using an N-terminal GST tag. The gene accession number is NM_152696.

Gene Aliases:

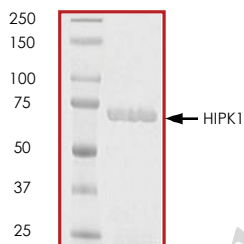
Myak, Nbak2, KIAA0630, MGC26642, MGC33446, MGC33548

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 71kDa.



Storage:

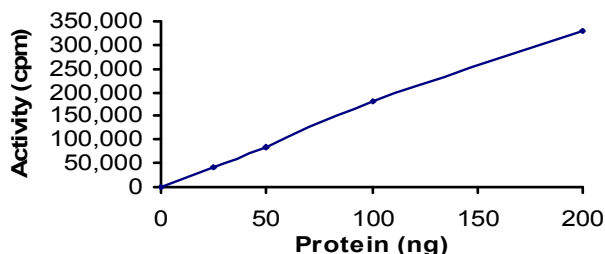
Store product at -70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

1 year at -70 °C from date of shipment.

**Specific Activity:**

The specific activity was determined to be 79 nmol /min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25 μ L reaction consisting of 5 μ L of 5 X Kinase Assay Buffer, 10 μ L of 1 mg/ml the Substrate Solution, 5 μ L of diluted kinase and 5 μ L of 250 μ M ATP solution containing [γ - 32 P] ATP (0.167 μ Ci/ μ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 μ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 1mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS pH 7.2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

- 1.Ecsedy, J A. et al: Homeodomain-interacting protein kinase 1 modulates Daxx localization, phosphorylation, and transcriptional activity. Mol Cell Biol. 2003 Feb;23(3):950-60.
- 2.Kondo, S. et al: Characterization of cells and gene-targeted mice deficient for the p53-binding kinase homeodomain-interacting protein kinase 1 (HIPK1). Proc Natl Acad Sci U S A. 2003 Apr 29;100(9):5431-6.

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