

## HIPK1, Active

Recombinant protein expressed in Sf9 cells

Catalog # H03-11G

Lot # P263-1

### 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.

### Storage and Stability

Store product at  $-70^{\circ}\text{C}$ . 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.

### Scientific Background

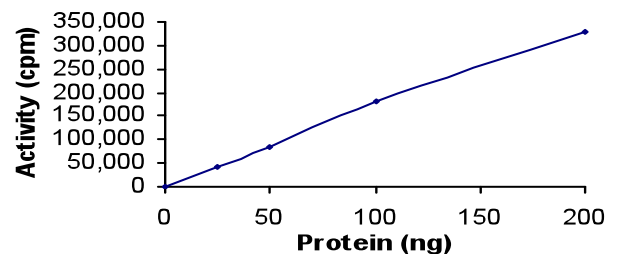
HIPK1 or homeodomain-interacting protein kinase 1 is a ser/thr protein kinase and a member of the HIPK family. HIPK1 is a nuclear kinase that phosphorylates homeodomain transcription factors. HIPK1 phosphorylates DAXX and this leads to its relocalization and subsequent decrease in transcriptional repression 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).

### 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.

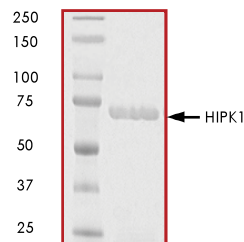
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### Specific Activity



The specific activity of HIPK1 was determined to be **79 nmol/min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>90%** by densitometry.  
Camk2 $\alpha$  Approx. MW **71kDa**.

## HIPK1, Active

Recombinant protein expressed in Sf9 cells

Catalog Number H03-11G

Specific Activity 79 nmol/min/mg

Specific Lot Number P263-1

Purity >90%

Concentration 0.1  $\mu\text{g}/\mu\text{l}$

Stability 1 yr at  $-70^{\circ}\text{C}$  from date of shipment

Storage & Shipping Store product at  $-70^{\circ}\text{C}$ . 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. Product shipped on dry ice.

# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: H03-11G)

Active HIPK1 (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active HIPK1 for optimal results).

### Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

### Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>32</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>32</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>32</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: M42-54G)

Myelin basic protein (MBP) diluted in distilled H<sub>2</sub>O to a final concentration of 0.2mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>32</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active HIPK1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1.** 10µl of diluted Active HIPK1 (Catalog #H03-11G)
  - Component 2.** 10µl of 0.2mg/ml stock solution of substrate (Catalog #M42-54G)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>32</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>32</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>32</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>32</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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