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Catalogue #	Aliquot Size
P33-20G-05	5 µg
P33-20G-10	10 µg

PTPN6 (SHP1), Active

Human recombinant protein expressed in E.coli cells

Catalog # P33-20G
Lot # W233-3

Product Description

Recombinant full length human PTPN6 was expressed in E.coli cells using an N-terminal GST tag. The gene accession number is NM_0080548.

Gene Aliases

SHP1; SHP-1; HCP; HCPH; HPTP1C; PTP-1C; SHP-1L; SH-PTP1

Formulation

Recombinant protein stored in 20mM MOPS, pH 7.5, 50mM NaCl, 10mM glutathione, 0.25mM DTT, 0.1mM PMSF, 30% glycerol.

Storage and Stability

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.

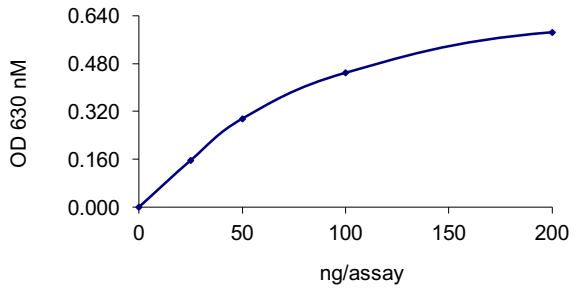
Scientific Background

PTPN6 gene is preferentially expressed in a variety of hematopoietic cells, and is an early response gene in lymphokine stimulated cells (1). The noncatalytic N-terminus of this PTP can interact with MAP kinases and negatively regulates ERK2 and p38 MAP-kinases activity (2). The PTPN6 was shown to be involved in the regulation of T cell antigen receptor (TCR) signaling, which was thought to function through dephosphorylating the molecules related to MAP kinase pathway.

References

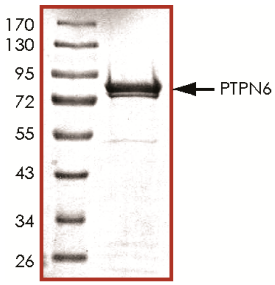
1. Adachi, M. et al: Protein-tyrosine phosphatase expression in pre-B cell NALM-6. *Cancer Res.* 52: 737-740, 1992.
2. Pettiford, S M. et al: The MAP-kinase ERK2 is a specific substrate of the protein tyrosine phosphatase HePTP. Oncogene. 2000 Feb 17;19(7):858-69.

Specific Activity



The specific activity of PTPN6 was determined to be **1100 nmol phosphate released /min/mg** as per activity assay protocol.

Purity



The purity of PTPN6 was determined to be **>95%** by densitometry. Approx. MW **93kDa**.

PTPN6 (SHP1), Active

Full-length human recombinant protein expressed in E.coli cells

Catalog Number	P33-20G
Specific Activity	1100 nmol/min/mg
Specific Lot Number	W233-3
Purity	>95%
Concentration	0.1µg/µl
Stability	1yr At –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
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Activity Assay Protocol

Reaction Components

Active Phosphatase (Catalog #: P33-20G)

Active PTPN6 (0.1μg/μl) diluted with Phosphatase Dilution Buffer II (Catalog #: P22-09) 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 PTPN6 for optimal results).

Phosphatase Dilution Buffer II (Catalog #: P22-09)

Phosphatase Assay Buffer II (Catalog #: P02-09) diluted at a 1:4 ratio (5X dilution) with freshly prepared solution containing 0.2% 2-mercaptolethanol and 65ng/μl BSA.

Phosphatase Assay Buffer II (Catalog #: P02-09)

Buffer components: 250 mM Imidazole, pH 7.2

Substrate Assay Solution (Catalog #: T70-58)

1 mM Tyrosine phosphopeptide-2 (DADEY(p)LIPDQG).

Detection Solution

BIOMOL GREEN reagent phosphatase detection kit (BioMol Catalog #: AK-111).

Assay Protocol

Step 1. Prepare a fresh batch of Phosphatase Dilution Buffer and keep on ice.

Step 2. Prepare phosphate standard curve following the instruction of BIOMOL GREEN reagent phosphatase detection kit. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with Phosphatase Dilution Buffer in a volume of 50μl. Also, use 50μl Phosphatase Dilution Buffer as a blank. The range of phosphate amount should be 0~4 nmol.

Step 3. Thaw the Active PTPN6 on ice. Prepare serial dilutions of PTPN6 using Phosphatase Dilution Buffer.

Step 4. In a pre-cooled microfuge tube, add the following reaction components in total volume of 50μl:

- **Component 1.** 10μl of diluted Active PTPN6 (Catalog #P33-20G)
- **Component 2.** 4μl of Substrate Assay solution (Catalog #T70-58)
- **Component 3.** 36μl Phosphatase Dilution Buffer II (Catalog #P22-09)

Step 5. Set up the blank control as outlined in step 4, excluding the addition of the Active Phosphatase. Replace the Active Phosphatase with an equal volume of Phosphatase Dilution Buffer (Catalog # P22-09).

Step 6. Start the reaction by incubating the mixture in a water bath at 37°C for 15 minutes.

Step 7. Add 100μl BIOMOL GREEN Reagent to each reaction including control tubes.

Step 8. Add 100μl BIOMOL GREEN Reagent to each phosphate standard solution including the blank (step 1).

Step 9. Incubate at room temperature for 30 minutes to allow development of the green color

Step 10. Measure the absorbance of the reaction solution in a spectrophotometer at 630 nm.

Step 11. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample – background absorbance) and calculate the corresponding nmol phosphate released (x) during the assay using the equation y = A*x + B or x =[y - B] / A (the A and B values are determined from the slope of the line from the standard curve).

Step 12. Calculate the phosphatase specific activity (SA):

Phosphatase Specific Activity (SA) (nmol/min/mg)

SA = Corresponding phosphate released *1000/ [(Reaction time in min)*(Enzyme amount in μg)]

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