3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

PTPN6, active, GST tagged, human recombinant, expressed in *E. coli* cells

Catalog Number **SRP5076**
Storage Temperature –70 °C

Synonyms: SHP1, SHP-1, HCP, HCPH, HPTP1C, PTP-1C, SHP-1L, SH-PTP1

Product Description
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] 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 the MAP kinase pathway.

Recombinant human PTPN6 was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_0080548. Recombinant protein stored in 20 mM MOPS, pH 7.5, 50 mM NaCl, 10 mM glutathione, 0.25 mM DTT, 0.1 mM PMSF, and 30% glycerol.

Molecular mass: ~93 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 820–1,110 nmol/min/mg (see Figure 2)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

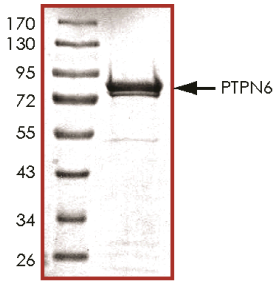
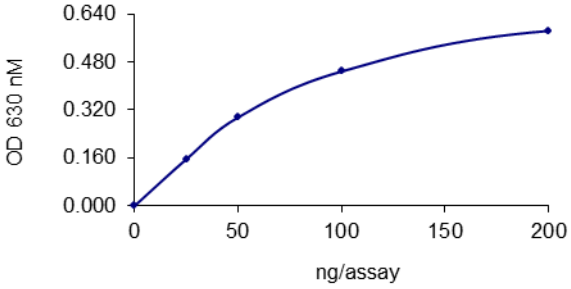


Figure 2.
Specific Activity of Typical Lot
820–1,110 nmol/min/mg



Procedure

Preparation Instructions

Phosphatase Assay Buffer- 250 mM Imidazole, pH 7.2

Phosphatase Dilution Buffer – Dilute phosphatase assay buffer 5-fold in a solution containing 0.2% 2-mercaptolethanol and 65 ng/µl BSA.

Phosphatase Solution – Dilute the active PTPN6 (0.1 µg/µl) with Phosphatase Dilution Buffer to the desired concentration.

Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PTPN6 for optimal results.

Substrate Assay Solution – 1 mM Tyrosine phosphopeptide-2 (DADE(pY)LIPQQG).

Detection Solution – BIOMOL Green™ Reagent (BioMol Cat. No. AK-111).

Phosphatase Assay

1. Prepare a fresh batch of Phosphatase Dilution Buffer and keep on ice.
2. Prepare phosphate standard curve following the instructions for BIOMOL Green Reagent. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with Phosphatase Dilution Buffer in a volume of 50 µl. Also, use 50 µl Phosphatase Dilution Buffer as a blank. The range of phosphate amount should be 0–4 nmole.
3. Thaw the active PTPN6 and Phosphate Dilution Buffer on ice. Prepare serial dilutions of PTPN6 using Phosphatase Dilution Buffer.
4. In a pre-cooled microcentrifuge tube, add the following reaction components in total volume of 50 µl:
   - 10 µl of Phosphatase Solution
   - 4 µl of Substrate Assay Solution
   - 36 µl of Phosphatase Dilution Buffer
5. Set up a blank control as outlined in step 4, substituting 10 µl of Phosphatase Dilution Buffer for the Phosphatase Solution.
6. Initiate each reaction by incubating the mixture in a water bath at 37 °C for 30 minutes.
7. Add 100 µl of BIOMOL Green Reagent to each reaction including control tubes.
8. Add 100 µl of BIOMOL Green Reagent to each phosphatase standard solution including blank.
9. Incubate all samples, controls, and standards at room temperature for 30 minutes to allow development of the green color.
10. Measure the absorbance of the reaction solution in a spectrophotometer at 630 nm.
11. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample-background absorbance) and calculate the corresponding nmole of 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).
12. Calculate the phosphatase specific activity (SA)

Calculations:

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

$$\text{nmole/min/mg} = \frac{x\ (1000)}{T \times E}$$

x - corresponding phosphate released
T - reaction time (min)
E - Enzyme amount (µg)

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, **19**, 858-869 (2000).

BIOMOL Green is a trademark of Enzo Life Sciences.

FF,MAM 11/11-1