

Product Information

PTPN11 (246-593), active, GST tagged, human recombinant, expressed in *E. coli* cells

Catalog Number **SRP5072**
Storage Temperature -70°C

Synonyms: SHP2, CFC, NS1, BPTP3, PTP2C, PTP-1D, SH-PTP2, SH-PTP3, MGC14433

Product Description

Mammalian PTPases can be subdivided into 1 of 2 broad categories: transmembrane receptor PTPases and intracellular PTPases. PTPN11 is one of the 2 closely related mammalian intracellular PTPases whose sequences encode 2 tandem SRC homology 2 (SH2) domains that are located on the amino-terminal side of a single PTPase catalytic domain.¹ This PTP is widely expressed in most tissues and plays a regulatory role in various cell signaling events that are important for a diversity of cell functions, such as mitogenic activation, metabolic control, transcription regulation, and cell migration.²

Recombinant human PTPN11 (246-593) was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_002834. 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: ~69 kDa

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

Specific Activity: 507–687 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)

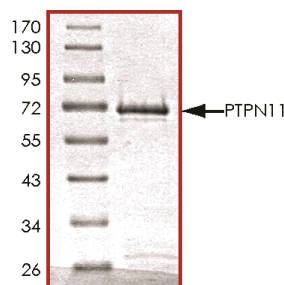
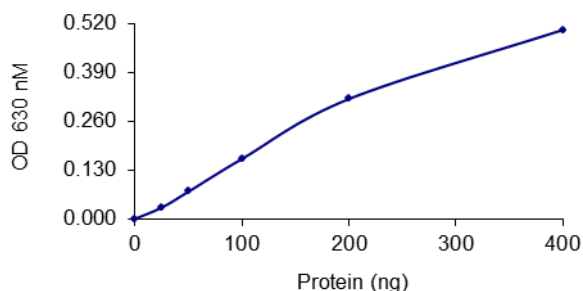


Figure 2.
Specific Activity of Typical Lot
507–687 nmol/min/mg



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-mercaptoethanol and 65 ng/μl BSA.

Phosphatase Solution – Dilute the active PTPN11 (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 PTPN11 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 PTPN11 and Phosphate Dilution Buffer on ice. Prepare serial dilutions of PTPN11 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

$$y = A \cdot x + B \text{ or } x = [y - B] / A$$

(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. Dechert, U. et al., Protein-tyrosine phosphatase SH-PTP2 (PTPN11) is localized to 12q24.1-24.3. Hum. Genet., **96**, 609-615 (1995).
2. Ahmad, S. et al., A widely expressed human protein-tyrosine phosphatase containing src homology 2 domains. Proc. Nat. Acad. Sci., **90**, 2197-2201 (1993).

BIOMOL Green is a trademark of Enzo Life Sciences.

FF,MAM 11/11-1