

## Product Information

**PTPN1, active, GST tagged, human recombinant, expressed in Sf9 cells**

Catalog Number **SRP5071**  
Storage Temperature  $-70^{\circ}\text{C}$

Synonym: PTP1B

### Product Description

Protein tyrosine phosphatase nonreceptor, type 1 (PTPN1) is the founding member of the protein tyrosine phosphatase (PTP) family.<sup>1</sup> PTPN1 catalyzes the hydrolysis of the phosphate monoesters specifically from tyrosine residues. PTPN1 is known to be involved in signaling pathways that regulate a variety of cellular processes including cell growth and oncogenic transformation. PTPN1 also has been shown to act as a negative regulator of insulin signaling.<sup>2</sup> PTPN1 has also been reported to dephosphorylate epidermal growth factor receptor kinase, which implicated the role of this phosphatase in cell growth control and cell response to interferon stimulation.

Recombinant full length human PTPN1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM\_002827. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, 25% glycerol.

Molecular mass: ~71 kDa

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

Specific Activity: 457–619 nmole/min/mg (see Figure 2)

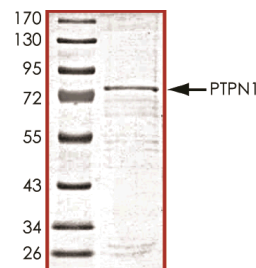
### Precautions and Disclaimer

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.

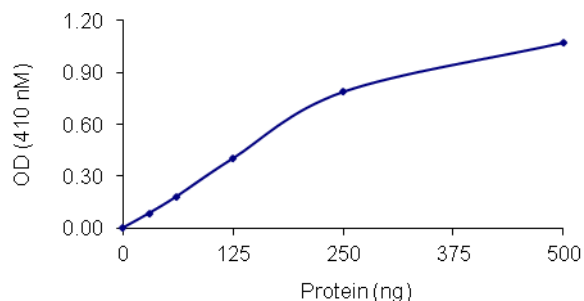
### Storage/Stability

The product ships on dry ice and storage at  $-70^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70^{\circ}\text{C}$ . 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  
457–619 nmole/min/mg



## Procedure

### Preparation Instructions

Phosphatase Assay Buffer – 125 mM HEPES, pH7.2, 250 mM NaCl, and 12.5 mM EDTA.

Phosphatase Dilution Buffer – Dilute the Phosphatase Assay Buffer 5-fold with a 5 mM DTT and 50 ng/μl BSA solution.

Phosphatase Solution – Dilute the active PTPN1 (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 PTPN1 for optimal results.

Stopping Solution – 2 M NaOH

Substrate Stock Solution – Prepare 50 mM *p*-nitrophenyl phosphate (pNPP) Substrate Stock Solution by dissolving 13.15 mg of pNPP in 1 ml of Phosphatase Dilution Buffer. Store at –20 °C. Avoid direct light exposure.

Substrate Assay Solution – Prepare 1.5 mM pNPP Substrate Assay Solution by diluting the Substrate Stock Solution at a 1:33.3 ratio with Phosphatase Dilution Buffer. Prepare fresh before assay.

### Phosphatase Assay

1. Prepare sufficient Substrate Assay Solution.
2. Thaw the active PTPN1 and Phosphate Dilution Buffer on ice.
3. In a pre-cooled microcentrifuge tube, add the following reaction components:
  - 10 μl of Phosphatase Solution
  - 50 μl of 1.5 mM pNPP Substrate Assay solution
  - 40 μl of Phosphatase Dilution Buffer
4. Set up a blank control as outlined in step 3, substituting 10μl of Phosphatase Dilution Buffer for the Phosphatase Solution.
5. Initiate each reaction by incubating the mixture in a water bath at 37 °C for 20 minutes.
6. After the 20 minute incubation, stop the reaction by the addition of 50 μl of 2 M NaOH Stopping Solution.
7. Measure the absorbance of the reaction solution in a spectrophotometer at 405 nm.
8. Determine the Phosphatase specific activity.

### Calculations:

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

$$\text{nmole/min/mg} = \frac{Pv \times OD_{405\text{nm}}}{\epsilon \times d \times T \times Pm}$$

- Pv - Phosphatase volume (μl)  
ε - extinction coefficient (17.8 μl/nmole/cm)  
d - pathlength of light (cm)  
T - incubation time (min)  
Pm - Phosphatase amount (mg)

### References

1. Cheyssac, C. et al., Analysis of common PTPN1 gene variants in type 2 diabetes, obesity and associated phenotypes in the French population. BMC Med. Genet., **5**, 44 (2006).
2. Bento, J.L. et al., Association of protein tyrosine phosphatase 1B gene polymorphisms with type 2 diabetes. Diabetes, **53**, 3007-12 (2004).

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