

Product Information

PTPN13 (2169-2485), active, GST tagged, human recombinant, expressed in *E. coli* cells

Catalog Number **SRP5074**
Storage Temperature -70°C

Synonyms: PTP-BAS, PNP1, FAP-1, PTP1E, PTPL1, PTPLE, PTP-BL, DKFZp686J1497

Product Description

PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. PTPN13 is a large protein that possesses a PTP domain at the C-terminus and multiple noncatalytic domains including a domain with similarity to band 4.1 superfamily of cytoskeletal-associated proteins, a region consisting of five PDZ domains, and a leucine zipper motif.¹ PTPN13 binds to a negative regulatory domain in Fas that inhibits Fas-induced apoptosis.²

Recombinant human PTPN13 (2169-2485) was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_080683. 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: ~61 kDa

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

Specific Activity: 2,040–2,760 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°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)

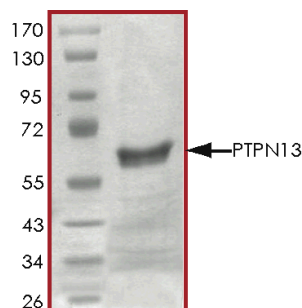
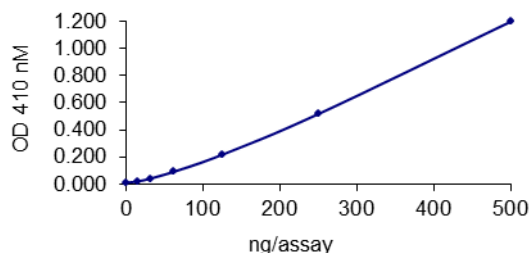


Figure 2.
Specific Activity of Typical Lot
2,040–2,760 nmole/min/mg



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 65 ng/μl BSA solution.

Phosphatase Solution – Dilute the active PTPN13 (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 PTPN13 for optimal results.

Stopping Solution – 2 M NaOH

Substrate Stock Solution – Prepare 500 mM *p*-nitro-phenyl phosphate (pNPP) Substrate Stock Solution by dissolving 131.5 mg of pNPP in 1 ml of Phosphatase Dilution Buffer. Store at –20 °C. Avoid direct light exposure.

Substrate Assay Solution – Prepare 50 mM pNPP Substrate Assay Solution by diluting the Substrate Stock Solution at a ten fold with Phosphatase Dilution Buffer. Prepare fresh before assay.

Phosphatase Assay

1. Prepare sufficient Substrate Assay Solution.
2. Thaw the active PTPN13 and Phosphate Dilution Buffer on ice.
3. In a pre-cooled microcentrifuge tube, add the following reaction components:
 - 10 μl of Phosphatase Solution
 - 20 μl of 50 mM pNPP Substrate Assay solution
 - 170 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:

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. Saras, J. et al., Cloning and characterization of PTPL1, a protein tyrosine phosphatase with similarities to cytoskeletal-associated proteins. J. Biol. Chem., **269**, 24082-24089 (1994).
2. Inazawa, J. et al., PTPN13, a Fas-associated protein tyrosine phosphatase, is located on the long arm of chromosome 4 at band q21.3. Genomics, **31**, 240-242 (1996).

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