

PTPN7 Phosphatase

5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
 This product is not intended for use as a therapeutic or in diagnostic procedures.

Description: Purified recombinant full-length human PTPN7 phosphatase, supplied as a GST fusion protein.

Background: Hematopoietic protein tyrosine phosphatase (HePTP, PTPN7) is a non-receptor protein tyrosine phosphatase that is expressed primarily in hematopoietic cells (1). The protein is made up of a short, amino-terminal extension that contains a conserved kinase interaction motif (KIM) and a carboxy-terminal protein tyrosine phosphatase domain (1,2). The presence of the kinase interaction motif allows PTPN7 to interact with MAPK family kinases, such as Erk1/2 and p38 MAP kinases (2,3). Reciprocal modification occurs between PTPN7 and Erk2, as the kinase phosphorylates PTPN7 at Thr45 and Ser72 while the phosphatase dephosphorylates Erk2 at Tyr185 (2). Down regulation of MAPK signaling follows removal of specific phospho-tyrosine residues from these MAPK kinases (3,4). Modification of MAPK family kinases by PTPN7 can regulate both kinase activity and localization (4).

Source/Purification: Recombinant full-length human PTPN7 (Met1-Pro360) was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_002832. The protein was purified by one-step affinity chromatography using glutathione-agarose.

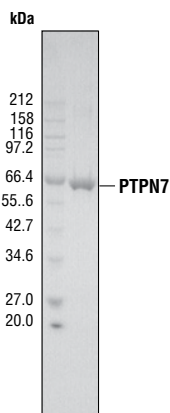


Figure 1. The purity of the GST-PTPN7 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-PTPN7 fusion protein is 66 kDa. The purified phosphatase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. PTPN7 phosphatase activity was determined using a DELFIA® assay [Fig.2].

Background References:

- (1) Adachi, M. et al. (1992) *Biochem Biophys Res Commun* 186, 1607-15.
- (2) Mustelin, T. et al. (2005) *J Mol Biol* 354, 150-63.
- (3) Muñoz, J.J. et al. (2003) *Biochem J* 372, 193-201.
- (4) Pettiford, S.M. and Herbst, R. (2003) *Leukemia* 17, 366-78.

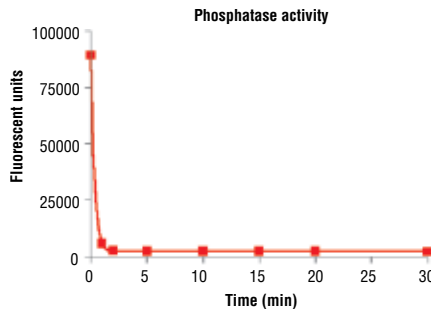


Figure 2. PTPN7 phosphatase activity was measured in a DELFIA® assay using the following reaction conditions: 25 mM HEPES, pH 7.2, 50 mM NaCl, 2.5 mM EDTA, 5 mM DTT, 65 ng/µl BSA. Substrate: Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586 at 1.5 µM, and 0.125 ng/µl PTPN7.

Entrez-Gene ID #5778
Swiss-Prot Acc. #P35236

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

- Poly (Glu-Tyr) Biotinylated Peptide #1585
- Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586
- Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

Protocol for Tyrosine Phosphatase

Phosphatase Assay:

A Additional Solutions and Reagents (Not included)

- **Phosphatase Buffer (5X)**
125 mM HEPES, pH 7.2
250 mM NaCl
12.5 mM EDTA
- Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586
- DTT (1.25 M)
- BSA (65 ng/μl)
- Stop solution (2N NaOH)
- Wash Buffer: 1X, PBS 0.05% Tween-20 (PBS/T)
- Phospho-Tyrosine mAb (P-Tyr-100) #9411

B Suggested Protocol for 100 Assays

1. Prepare fresh batches of 1X Phosphatase Assay Buffer by diluting the Phosphatase Buffer (5X) at a 1:4 ratio with a solution containing 5 mM DTT and 65 ng/μl BSA.
2. Dilute 1 mM Phospho-Poly EY (20) Biotinylated Peptide substrate solution to 3 μM with 1X Phosphatase Assay Buffer.
3. Thaw enzyme on ice.
4. Dilute phosphatase protein to 0.2 to 2.0 ng/μl with 1X Phosphatase Assay Buffer.
5. To start the reaction combine 25 μl diluted phosphatase solution and 25 μl substrate (3 μM). Incubate at 37°C for 5 to 60 minutes.

Final Assay Conditions for a 50 μl Reaction

- 25 mM HEPES, pH 7.2
 - 50 mM NaCl
 - 2.5 mM EDTA
 - 5 mM DTT
 - 65 ng/μl BSA
 - 1.5 μM Phospho-Poly (Glu-Tyr) Biotinylated Peptide
 - 0.1 to 1.0 ng/μl phosphatase
6. Terminate reaction by adding 50 μl of 2N NaOH Stop Solution to each reaction well.
 7. For DELFIA® or colorimetric ELISA detection methods please use the protocols described to the right.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
 2. Add 100 μl/well secondary antibody solution.
 3. Incubate at room temperature for 30 minutes.
 4. *Wash five times with 200 μl/well PBS/T.
 5. Add 100 μl/well DELFIA® Enhancement Solution.
 6. Incubate at room temperature for 5 minutes.
 7. Read plate using a time resolved fluorescent plate reader using the following settings;
 - a. Excitation Filter: 340 nm
 - b. Emission Filter: 615 nm
 - c. Delay**: 400 μs
- ** Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
 DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
 DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
 DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 μl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 μl/well PBS/T.
5. Add 100 μl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 μl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
 Anti-rabbit IgG, HRP Linked Antibody #7074
 TMB Solution #7004
 Stop Solution #7002

* **NOTE:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

For any questions please contact:

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