#8008 Store at –80°C

PTPN13 Phosphatase

☑5 µg

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New 04/08

Entrez-Gene ID #5783
Swiss-Prot Acc. #Q12923

Description: Purified recombinant human PTPN13 (Asp2169-Lys2485) phosphatase, supplied as a GST fusion protein.

Background: Non-receptor protein tyrosine phosphatase 13 (PTPN13) is also known as Fas-associated phosphatase-1 (FAP-1) because it associates with the regulatory domain of the Fas receptor and negatively regulates Fas signaling (1). PTPN13 protein contains: an N-terminal kinase non-catalytic C-lobe domain (KIND); followed by a Four-point-one/Ezrin/Radixin/Moesin (FERM) domain; five PDZ domains; and a C-terminal phosphatase domain (2,3). The function of the KIND domain is not clear, while the FERM domain is thought to bind cell surface receptors and help target PTPN13 to the plasma membrane. The PDZ domains may help PTPN13 bind selectively to target proteins and maintain substrate specificity (2). Dephosphorylation of Fas receptor by PTPN13 reduces the presence of the receptor at the cell surface and inhibits Fas-mediated apoptosis (4). Both Fas receptor and its inhibitor are regulated through NF-κB signaling. Activated Fas receptor induces NF-κB signaling, which promotes expression of the Fas inhibitor PTPN13 (5). Induced overexpression of PTPN13 *in vitro* blocks IRS-1/PI3K/Akt signaling by dephosphorylating IRS-1, leading to reduced cell survival and induction of apoptosis (6). Mutations in the corresponding PTPN13 gene and abnormal phosphatase activity are associated with colorectal cancer, suggesting that PTPN13 plays an important role in regulating cancer cell proliferation (7). Similar studies indicate that PTPN13 may act as a tumor suppressor in hepatocellular carcinoma as inhibition of PTPN13 phosphatase activity results in increased cancer cell proliferation (8).

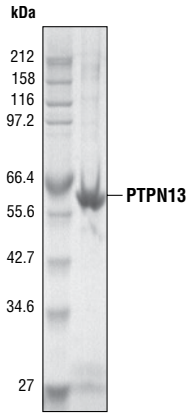

Figure 1. The purity of the PTPN13 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-phosphatase fusion protein was produced by expressing recombinant human PTPN13 (Asp2169-Lys2485) (GenBank Accession No. NM_080683) with an amino-terminal GST tag in *E. coli*. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-PTPN13 fusion protein is 61 kDa. The purified phosphatase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. PTPN13 phosphatase activity was determined using a DELFIA® assay [Fig.2].

Background References:

(1) Sato, T. et al. (1995) *Science* 268, 411–5.
(2) Erdmann, K.S. (2003) *Eur J Biochem* 270, 4789–98.
(3) Saras, J. et al. (1994) *J Biol Chem* 269, 24082–9.
(4) Ivanov, V.N. et al. (2003) *Mol Cell Biol* 23, 3623–35.
(5) Ivanov, V.N. et al. (2006) *J Biol Chem* 281, 1840–52.
(6) Dromard, M. et al. (2007) *Cancer Res* 67, 6806–13.
(7) Wang, Z. et al. (2004) *Science* 304, 1164–6.
(8) Yeh, S.H. et al. (2006) *Clin Cancer Res* 12, 1097–108.

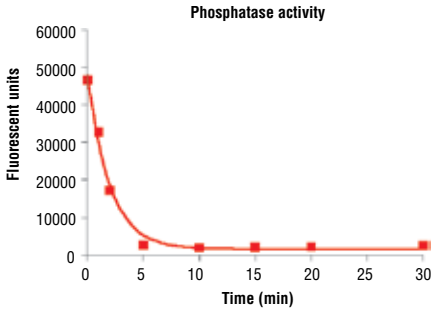

Figure 2. PTPN13 phosphatase activity was measured in a DELFIA® assay using the following reaction conditions: 25 mM HEPES, pH 7.2, 50 mM NaCl, 2.5 mM EDTA, 5 mM DTT, 65 ng/µl BSA, Substrate: Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586 at 1.5 µM, and 0.06 ng/µl PTPN13.

Storage: Enzyme is supplied in 20 mM MOPS, pH7.5; 50 mM NaCl, 0.25 mM DTT, 0.1 mM PMSF, 30% 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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