Store at –80°C

#8001

# PTPα Phosphatase

☑5 µg

Cell Signaling TECHNOLOGY®

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New 04/08



**Description:** Purified recombinant human PTPα (Met174-Lys802) phosphatase, supplied as a GST fusion protein.

**Background:** PTPα (PTPRA) is a transmembrane receptor tyrosine phosphatase implicated in the regulation of Src family kinases during the G2 to mitosis entry point. Two identified splice variants differ in the size of the extracellular region; the shorter form appears to be ubiquitously expressed while the larger protein is more limited in distribution (1). The cytoplasmic region of PTPα contains two putative catalytic domains. One phosphatase domain (D1) exhibits catalytic activity while the other (D2) may regulate phosphatase activity by allowing receptor dimer formation (2,3). PTPα is a physiological regulator of Src and Src family kinases (4). Constitutive phosphorylation of the carboxy-terminal Tyr789 of PTPα is essential for dephosphorylation of Src at Tyr527. Phosphorylation of PTPα at this residue also allows binding of the Grb2 inhibitor, restricting PTPα activation of Src (5,6). PKC-mediated phosphorylation of the PTP at Ser180 and Ser204 also increases PTPα phosphatase activity (7).

**Source/Purification:** The GST-phosphatase fusion protein was produced by expressing recombinant human PTPα (Met174-Lys802) (GenBank Accession No. NM_002836) with an amino-terminal tag in *E. coli*. The protein was purified by one-step affinity chromatography using glutathione-agarose.

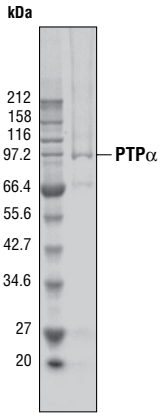


*Figure 1. The purity of the PTPRA fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.*

**Quality Control:** The theoretical molecular weight of the GST-PTPα fusion protein is 96 kDa. The purified phosphatase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. PTPα phosphatase activity was determined using a DELFIA® assay [Fig.2].

**Background References:**

(1) Kapp, K. et al. (2007) *Genes Cells* 12, 63–73.
(2) Blanchetot, C. et al. (2002) *J Biol Chem* 277, 47263–9.
(3) Krueger, N.X. et al. (1990) *EMBO J* 9, 3241–52.
(4) den Hertog, J. et al. (1993) *EMBO J* 12, 3789–98.
(5) Zheng, X.M. et al. (2000) *EMBO J* 19, 964–78.
(6) Zheng, X.M. et al. (2002) *J Biol Chem* 277, 21922–9.
(7) Tracy, S. et al. (1995) *J Biol Chem* 270, 10587–94.

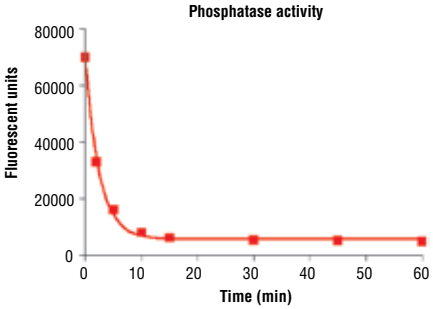


*Figure 2. PTPα phosphatase activity was measured in a DELFIA® assay using the following reaction conditions: 25 mM HEPES, pH 7.2, 50 mM NaCl, 2.5 mM EDTA, 5 mM DTT, 65 ng/µl BSA, Substrate: Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586 at 1.5 µM, and 0.25 ng/µl PTPα.*

**Entrez-Gene ID** #5786
**Swiss-Prot Acc.** #P18433

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1mM 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:**
Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

Poly (Glu-Tyr) Biotinylated Peptide #1585

Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586

# 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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