

PTPRF Phosphatase

✓ 5 µg



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New 04/08

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 human PTPRF (Ile1275-Glu1897) phosphatase, supplied as a GST fusion protein.

Background: Receptor type protein tyrosine phosphatase F (PTPRF, LAR) is a transmembrane PTP that helps to regulate insulin signaling, cell proliferation and cell migration. The PTPRF protein is composed of an extracellular segment that contains several Ig-like and fibronectin (Fn-III) domains, a transmembrane region and a pair of cytoplasmic phosphatase domains (1,2). Functional studies reveal that the membrane-associated D1 phosphatase domain is responsible for substrate dephosphorylation, while the D2 domain is important for substrate specificity (3). PTPRF negatively regulates insulin signaling through dephosphorylation of insulin receptor and insulin receptor substrate (4). This phosphatase activates the pro-apoptotic DAPK serine/threonine kinase by removing a phosphate at Tyr491/492, while the kinase Src replaces the phosphate to inactivate DAPK at the same time it down regulates PTPRF expression (5). PTPRF is commonly found at focal adhesions where it interacts with liprin, which localizes the phosphatase to the membrane, and the Rac/Rho family GTPase Trio (6). Localization of PTPRF at adherens junctions results in PTPRF modification of β -catenin, which inhibits cell migration by limiting the amount of available cytosolic β -catenin (7).

Source/Purification: The GST-phosphatase fusion protein was produced by expressing recombinant human PTPRF (Ile1275-Glu1897)(GenBank Accession No. NM_002840) with an amino-terminal tag in *E. coli*. The protein was purified by one-step affinity chromatography using glutathione-agarose.

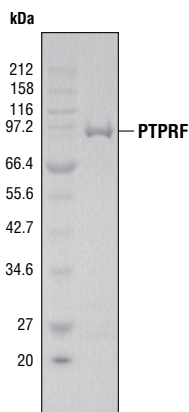


Figure 1. The purity of the GST-PTPRF fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-PTPRF fusion protein is 93 kDa. The purified phosphatase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. PTPRF phosphatase activity was determined using a DELFIA® assay [Fig.2].

Background References:

- (1) Cheng, A. et al. (2002) *Eur J Biochem* 269, 1050–9.
- (2) O'Grady, P. et al. (1994) *J Biol Chem* 269, 25193–9.
- (3) Tsujikawa, K. et al. (2001) *Mol Endocrinol* 15, 271–80.
- (4) Zhang, W.R. et al. (1996) *Mol Endocrinol* 10, 575–84.
- (5) Wang, W.J. et al. (2007) *Mol Cell* 27, 701–16.
- (6) Stoker, A.W. (2005) *J Endocrinol* 185, 19–33.
- (7) Müller, T. et al. (1999) *J Biol Chem* 274, 10173–83.

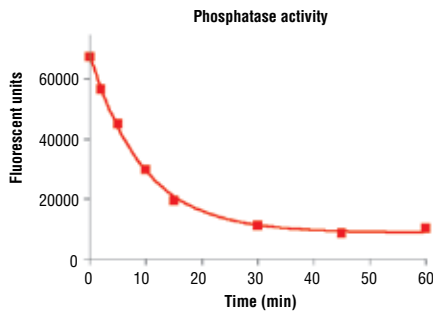


Figure 2. PTPRF phosphatase activity was measured in a DELFIA® assay using the following reaction conditions: 25 mM HEPES, pH 7.2, 50 mM NaCl, 2.5 mM EDTA, 5 mM DTT, 65 ng/µl BSA, Substrate: Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586 at 1.5 µM, and 0.125 ng/µl PTPRF.

Entrez-Gene ID #5792
Swiss-Prot Acc. #P10586

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:

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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