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Product Information

PTPRF (1275-1897), active, GST tagged, human recombinant, expressed in *E. coli* cells

Catalog Number **SRP5080**
Storage Temperature –70 °C

Synonym: LAR

Product Description
PTPRF or LAR is a member of the protein tyrosine phosphatase family with an extracellular region, a single transmembrane region, and two tandem intracytoplasmic catalytic domains.[1] PTPRF has been shown to function in the regulation of epithelial cell-cell contacts at adherents junctions as well as in the control of β-catenin signaling. An increased expression level of this protein was found in the insulin-responsive tissue of obese, insulin-resistant individuals and may contribute to the pathogenesis of insulin resistance.[2]

Recombinant human PTPRF (1275-1897) was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_002840. 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: ~93 kDa

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

Specific Activity: 386–522 nmol/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)

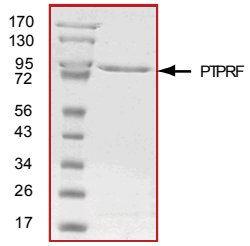
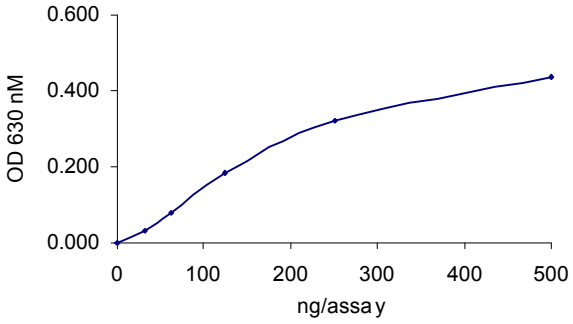


Figure 2.
Specific Activity of Typical Lot
386–522 nmol/min/mg



Procedure

Preparation Instructions

Phosphatase Assay Buffer- 250 mM Imidazole, pH 7.2

Phosphatase Dilution Buffer – Dilute phosphatase assay buffer 5-fold in a solution containing 0.2% 2-mercaptolethanol and 65 ng/µl BSA.

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

Substrate Assay Solution – 1 mM Tyrosine phosphopeptide-2 (DADE(pY)LIPQQG).

Detection Solution – BIOMOL Green™ Reagent (BioMol Cat. No. AK-111).

Phosphatase Assay

1. Prepare a fresh batch of Phosphatase Dilution Buffer and keep on ice.
2. Prepare phosphate standard curve following the instructions for BIOMOL Green Reagent. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with Phosphatase Dilution Buffer in a volume of 50 µl. Also, use 50 µl Phosphatase Dilution Buffer as a blank. The range of phosphate amount should be 0–4 nmole.
3. Thaw the active PTPRF and Phosphate Dilution Buffer on ice. Prepare serial dilutions of PTPRF using Phosphatase Dilution Buffer.
4. In a pre-cooled microcentrifuge tube, add the following reaction components in total volume of 50 µl:
   - 10 µl of Phosphatase Solution
   - 4 µl of Substrate Assay Solution
   - 36 µl of Phosphatase Dilution Buffer
5. Set up a blank control as outlined in step 4, substituting 10 µl of Phosphatase Dilution Buffer for the Phosphatase Solution.
6. Initiate each reaction by incubating the mixture in a water bath at 37 °C for 30 minutes.
7. Add 100 µl of BIOMOL Green Reagent to each reaction including control tubes.
8. Add 100 µl of BIOMOL Green Reagent to each phosphatase standard solution including blank.
9. Incubate all samples, controls, and standards at room temperature for 30 minutes to allow development of the green color.
10. Measure the absorbance of the reaction solution in a spectrophotometer at 630 nm.
11. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample-background absorbance) and calculate the corresponding nmole of phosphate released (x) during the assay using the equation

    $y = A*x + B$ or $x = [y-B]/A$
    (the A and B values are determined from the slope of the line from the standard curve).
12. Calculate the phosphatase specific activity (SA)

Calculations:

Specific Phosphatase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{x\ (1000)}{T \times E}$$

x - corresponding phosphate released
T - reaction time (min)
E - Enzyme amount (µg)

References

1. Ahmad, F. et al., Functional association between the insulin receptor and the transmembrane protein-tyrosine phosphatase LAR in intact cells. J. Biol. Chem., **272**, 448-457 (1997).
2. Tsujikawa, K. et al., Distinct functions of the two protein tyrosine phosphatase domains of LAR (leukocyte common antigen-related) on tyrosine dephosphorylation of insulin receptor. Molec. Endocr., **15**, 271-280, (2001).

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