

Product Information

PTPRC (592-end), active, GST tagged, mouse recombinant, expressed in *E. coli* cells

Catalog Number **SRP5079**
Storage Temperature -70°C

Synonyms: B220, Cd45, CD45R, loc, Ly-5, Lyt-4, T200

Product Description

PTPRC (CD45) contains an extracellular domain, a single transmembrane segment, and two tandem intracytoplasmic catalytic domains, and thus belongs to receptor type PTP.¹ This gene is specifically expressed in hematopoietic cells.² PTPRC has been shown to be an essential regulator of T and B cell antigen receptor signaling. It functions through either direct interaction with components of the antigen receptor complexes or by activating various Src family kinases required for the antigen receptor signaling.

Recombinant mouse PTPRC (592-end) was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_001111316. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~116 kDa

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

Specific Activity: 8,415–11,385 nmole/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)

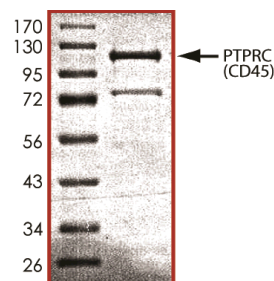
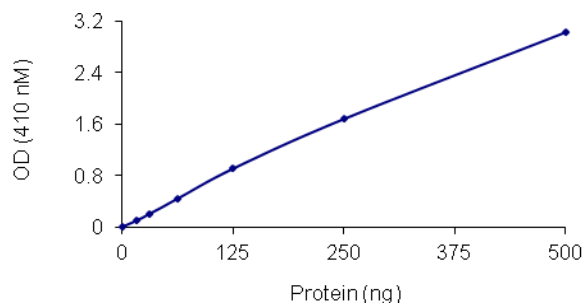


Figure 2.
Specific Activity of Typical Lot
8,415–11,385 nmole/min/mg



Procedure

Preparation Instructions

Phosphatase Assay Buffer – 125 mM HEPES, pH7.2, 250 mM NaCl, and 12.5 mM EDTA.

Phosphatase Dilution Buffer – Dilute the Phosphatase Assay Buffer 5-fold with a 5 mM DTT and 65 ng/μl BSA solution.

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

Stopping Solution – 2 M NaOH

Substrate Stock Solution – Prepare 500 mM *p*-nitrophenyl phosphate (pNPP) Substrate Stock Solution by dissolving 131.5 mg of pNPP in 1 ml of Phosphatase Dilution Buffer. Store at –20 °C. Avoid direct light exposure.

Substrate Assay Solution – Prepare 50 mM pNPP Substrate Assay Solution by diluting the Substrate Stock Solution 10-fold with Phosphatase Dilution Buffer. Prepare fresh before assay.

Phosphatase Assay

1. Prepare sufficient Substrate Assay Solution.
2. Thaw the active PTPRC and Phosphate Dilution Buffer on ice.
3. In a pre-cooled microcentrifuge tube, add the following reaction components:
 - 10 μl of Phosphatase Solution
 - 20 μl of 50 mM pNPP Substrate Assay solution
 - 170 μl of Phosphatase Dilution Buffer
4. Set up a blank control as outlined in step 3, substituting 10μl of Phosphatase Dilution Buffer for the Phosphatase Solution.
5. Initiate each reaction by incubating the mixture in a water bath at 37 °C for 20 minutes.
6. After the 20 minute incubation, stop the reaction by the addition of 50 μl of 2 M NaOH Stopping Solution.
7. Measure the absorbance of the reaction solution in a spectrophotometer at 405 nm.
8. Determine the Phosphatase specific activity.

Calculations:

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

$$\text{nmole/min/mg} = \frac{Pv \times OD_{405\text{nm}}}{\epsilon \times d \times T \times Pm}$$

- Pv - Phosphatase volume (μl)
ε - extinction coefficient (17.8 μl/nmole/cm)
d - pathlength of light (cm)
T - incubation time (min)
Pm - Phosphatase amount (mg)

References

1. Ralph, S.J., et al. Structural variants of human T200 glycoprotein (leukocyte-common antigen). *EMBO J.*, **6**, 1251-1257 (1987).
2. Fischer, E.H., et al. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science*, **253**, 401-406 (1991).

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