

PTPRC (CD45), Active

Recombinant human protein expressed in Sf9 cells

Catalog # P50-21H

Lot # R003-2

Product Description

Recombinant human PTPRC (604-end) was expressed in Sf9 insect cells using an N-terminal His tag. The gene accession number is [NM_002838](#).

Gene Aliases

CD45, LCA, T200, LY5, B220, GP180.

Formulation

Recombinant protein stored in 50mM MOPS, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol.

Storage and Stability

Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

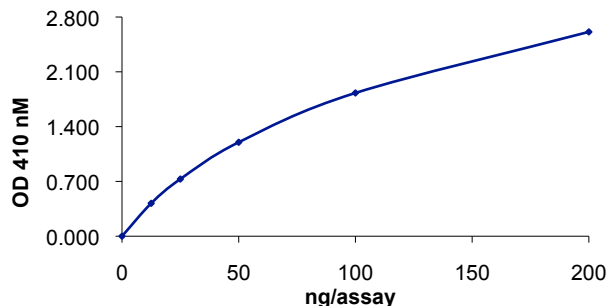
Scientific Background

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.

References

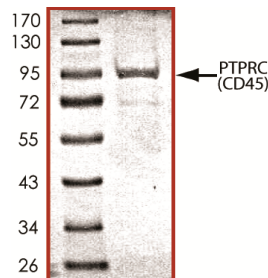
- Ralph, S. J. et al: Structural variants of human T200 glycoprotein (leukocyte-common antigen). *EMBO J.* 6: 1251-1257, 1987.
- Fischer, E. H. et al: Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science* 253: 401-406, 1991.

Specific Activity



The specific activity of PTPRC was determined to be **11,500 nmol phosphate released /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **95kDa**.

PTPRC (CD45), Active

Recombinant protein expressed in Sf9 cells

Catalog Number	P50-21H
Specific Activity	11,500 nmol/min/mg
Specific Lot Number	R003-2
Purity	>90%
Concentration	0.1µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Phosphatase (Catalog #: P50-21H)

Active PTPRC (0.1µg/µl) diluted with Phosphatase Dilution Buffer I (Catalog #: P21-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PTPRC for optimal results).

Phosphatase Dilution Buffer I (Catalog #: P21-09)

Phosphatase Assay Buffer I (Catalog #: P01-09) diluted at a 1:4 ratio (5X dilution) with a solution containing 5 mM DTT and 65 ng/µl BSA.

Phosphatase Assay Buffer I (Catalog #: P01-09)

Buffer components: 125 mM HEPES pH 7.2, 250 mM NaCl, 12.5 mM EDTA.

Substrate Assay Solution

Prepare 50 mM pNPP Substrate Assay Solution by diluting the Substrate Stock Solution at a 1:9 ratio (10X dilution) with Phosphatase Dilution Buffer I (Catalog #: P21-09). Prepare fresh before assay.

Substrate Stock Solution

Prepare 500 mM p-nitrophenyl phosphate (pNPP) Substrate Stock Solution by dissolving 131.5 g pNPP in 1 ml of Phosphatase Dilution Buffer I (Catalog #: P21-09). Store at -20°C. Avoid direct light exposure.

Stopping Solution

2M NaOH.

Assay Protocol

- Step 1.** Prepare fresh batches of Phosphatase Dilution Buffer and Substrate Assay Solution. Keep them cool on ice.
- Step 2.** Thaw the Active PTPRC on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components in total volume of 200µl:

Component 1. 10µl of diluted Active PTPRC (Catalog #P50-21H)

Component 2. 20µl of 50 mM PNPP Substrate Assay solution

Component 3. 170µl Phosphatase Dilution Buffer I (Catalog #P21-09)

- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the Active Phosphatase. Replace the Active Phosphatase with an equal volume of Phosphatase Dilution Buffer (Catalog #P21-09).
- Step 5.** Start the reaction by incubating the mixture in a water bath at 37°C for 10 minutes.
- Step 6.** After the 10 minute incubation period, terminate the reaction by the addition of 50 µl of 2M NaOH Stopping Solution.
- Step 7.** Measure the absorbance of the reaction solution in a spectrophotometer at 405 nm.
- Step 8.** Determine the Phosphatase specific activity as outlined below.

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

$$SA = \frac{[\text{volume of Phosphatase used } (\mu\text{l}) * OD_{405\text{nm}}]}{[\text{extinction coefficient} * \text{incubation time (min)} * \text{pathlength of light (cm)} * \text{Phosphatase amount in mg}]}$$

The extinction coefficient is 17.8 µl/nmol/cm

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