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Catalogue #	Aliquot Size
P39-21G-05	5 µg
P39-21G-10	10 µg
P39-21G-20	20 µg

PTPN12 (PTP-PEST), Active

Human recombinant protein expressed in E.coli cells

Catalog # P39-21G
Lot # C150-1

Product Description

Recombinant human PTPN12 (1-355) was expressed in E.coli cells using an N-terminal GST tag. The gene accession number is NM_002835.

Gene Aliases

PTP-PEST, PTPG1, tcag7.1075

Formulation

Recombinant protein stored in 20mM MOPS, pH 7.5, 50mM NaCl, 10mM glutathione, 0.25mM DTT, 0.1mM PMSF, 30% 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.

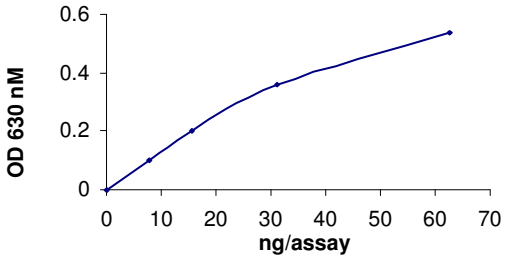
Scientific Background

Protein tyrosine phosphatase-PEST (PTPN12), a ubiquitously expressed cytoplasmic tyrosine phosphatase, is thought to play an important role in cell adhesion and motility, cell migration, and signal transduction for antigen receptors in B and T lymphocytes (1). Signal transduction via tyrosine phosphorylation, normally fine-tuned by the concerted action of both protein tyrosine kinases and protein tyrosine phosphatases (PTPs), is a key mechanism in tumorigenesis. Studies suggest potential role for PTP-PEST in regulation of p130(cas) in mitogen- and cell adhesion-induced signaling events (2).

References

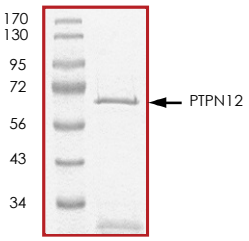
1. Angers-Loustau, A. et al: Protein tyrosine phosphatase-PEST regulates focal adhesion disassembly, migration, and cytokinesis in fibroblasts. J. Cell Biol. 1999; 144**:** 1019-31.
2. Garton, A.J. et al: Identification of p130(cas) as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. Mol. Cell Biol. 1996; 16(11):6408-18.

Specific Activity



The specific activity of PTPN12 was determined to be **2965 nmol phosphate released /min/mg** as per activity assay protocol.

Purity



The purity of PTPN12 was determined to be **>75%** by densitometry. Approx. MW **66kDa**.

PTPN12 (PTP-PEST), Active

Recombinant protein expressed in E.coli cells

Catalog Number	P39-21G
Specific Activity	2965 nmol/min/mg
Specific Lot Number	C150-1
Purity	>75%
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 #: P39-21G)

Active PTPN12 (0.1µg/µl) diluted with Phosphatase Dilution Buffer II (Catalog #: P22-09) 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 PTPN12 for optimal results).

Phosphatase Dilution Buffer II (Catalog #: P22-09)

Phosphatase Assay Buffer II (Catalog #: P02-09) diluted at a 1:4 ratio (5X dilution) with freshly prepared solution containing 0.2% 2-mercaptolethanol and 65ng/µl BSA.

Phosphatase Assay Buffer II (Catalog #: P02-09)

Buffer components: 250 mM Imidazole, pH 7.2

Substrate (Catalog #: T70-58)

1 mM Tyrosine phosphopeptide-2 (DADE(pY)LIPQQG) solution.

Detection Solution

BIOMOL GREEN reagent phosphatase detection kit (BioMol Catalog #: AK-111).

Assay Protocol

Step 1. Prepare a fresh batch of Phosphatase Dilution Buffer and keep on ice.

Step 2. Prepare phosphate standard curve following the instruction of BIOMOL GREEN reagent phosphatase detection kit. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with Phosphatase Dilution Buffer in a volume of 50µl. Also, use 50µl Phosphatase Dilution Buffer as a blank. The range of phosphate amount should be 0~4 nmol.

Step 3. Thaw the Active PTPN12 on ice. Prepare serial dilutions of PTPN12 using Phosphatase Dilution Buffer.

Step 4. In a pre-cooled microfuge tube, add the following reaction components in total volume of 50µl:

Component 1. 10µl of diluted Active PTPN12 (Catalog #P39-21G)

Component 2. 4µl of 1 mM Tyrosine phosphopeptide-2 Substrate (Catalog #T70-58)

Component 3. 36µl Phosphatase Dilution Buffer II (Catalog #P22-09)

Step 5. Set up the blank control as outlined in step 4, excluding the addition of the Active Phosphatase. Replace the Active Phosphatase with an equal volume of Phosphatase Dilution Buffer (Catalog # P22-09).

Step 6. Start the reaction by incubating the mixture in a water bath at 37°C for 20 minutes.

Step 7. Add 100µl BIOMOL GREEN Reagent to each reaction including control tubes.

Step 8. Add 100µl BIOMOL GREEN Reagent to each phosphate standard solution including the blank (step 1).

Step 9. Incubate at room temperature for 30 minutes to allow development of the green color

Step 10. Measure the absorbance of the reaction solution in a spectrophotometer at 630 nm.

Step 11. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample – background absorbance) and calculate the corresponding nmol 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).

Step 12. Calculate the phosphatase specific activity (SA):

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

SA = Corresponding phosphate released *1000/ [(Reaction time in min)*(Enzyme amount in µg)]

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