

PTPN11 (SHP2), Active

Human recombinant protein expressed in E.coli cells

Catalog # P38-21G

Lot # V2575-4

Product Description

Recombinant human PTPN11 (246-593) was expressed in E.coli cells using an N-terminal GST tag. The gene accession number is [NM_002834](#).

Gene Aliases

SHP2, CFC, NS1, BTP3, PTP2C, PTP-1D, SH-PTP2, SH-PTP3, MGC14433

Formulation

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

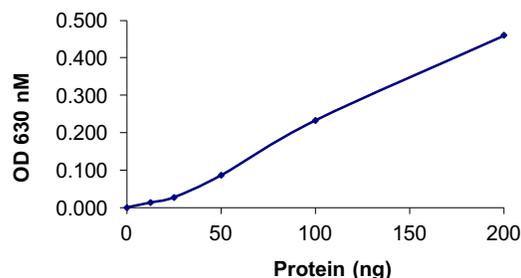
Scientific Background

Mammalian PTPases can be subdivided into 1 of 2 broad categories: transmembrane receptor PTPases and intracellular PTPases. PTPN11 is one of the 2 closely related mammalian intracellular PTPases whose sequences encode 2 tandem SRC homology 2 (SH2) domains that are located at the amino-terminal side of a single PTPase catalytic domain (1). This PTP is widely expressed in most tissues and plays a regulatory role in various cell signaling events that are important for a diversity of cell functions, such as mitogenic activation, metabolic control, transcription regulation, and cell migration (2).

References

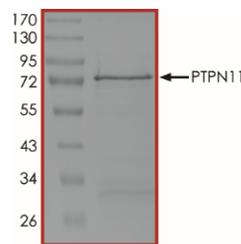
1. Dechert, U. et al: Protein-tyrosine phosphatase SH-PTP2 (PTPN11) is localized to 12q24.1-24.3. *Hum. Genet.* 96: 609-615, 1995.
2. Ahmad, S. et al: A widely expressed human protein-tyrosine phosphatase containing src homology 2 domains. *Proc. Nat. Acad. Sci.* 90: 2197-2201, 1993.

Specific Activity



The specific activity of PTPN11 was determined to be **649.3 nmol phosphate released /min/mg** as per activity assay protocol.

Purity



The purity of PTPN11 was determined to be **>90%** by densitometry. Approx. MW **69kDa**.

PTPN11 (SHP2), Active

Human recombinant protein expressed in E.coli cells

Catalog #	P38-21G
Specific Activity	649.3 nmol/min/mg
Lot #	V2575-4
Purity	>90%
Concentration	0.05µg/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 #: P38-21G)

Active PTPN11 (0.05µg/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 PTPN11 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-mercaptoethanol and 65ng/µl BSA.

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

Buffer components: 250 mM Imidazole, pH 7.2

Substrate Assay Solution (Catalog #: T70-58)

1 mM Tyrosine phosphopeptide-2 (DADEY(p)LIPDQG).

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. As a blank, use 50µl Phosphatase Dilution Buffer. The range of phosphate amount should be 0~4 nmol.
- Step 3.** Thaw the Active PTPN11 on ice. Prepare serial dilutions of PTPN11 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 PTPN11 (Catalog #P38-21G)

Component 2. 4µl of Substrate Assay Solution (Catalog #T70-58)

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

- Step 5.** 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 # P22-09).
- Step 6.** Start the reaction by incubating the mixture in a water bath at 37°C for 15 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)

$$SA = \text{Corresponding phosphate released} * 1000 / [(\text{Reaction time in min}) * (\text{Enzyme amount in } \mu\text{g})]$$

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