

Certificate of Analysis

PTPN22, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-768, 14-768-K, 14-768M

Parent Lot # D7BN019N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal GST-tagged, recombinant, human PTPN22, amino acids 1–312, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 63.3kDa.

Specific Activity (Parent lot# D7BN019N): 12806U/mg, where one unit of PTPN22 activity is defined as the release of 1nmol of phosphate per minute from the phosphorylated substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) at room temperature.

Formulation: 0.43mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

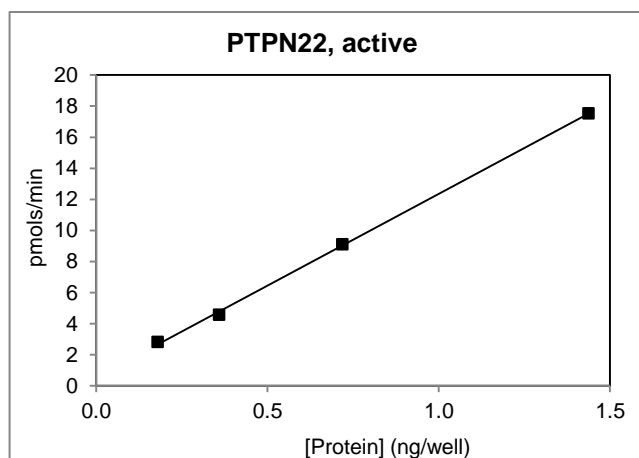
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

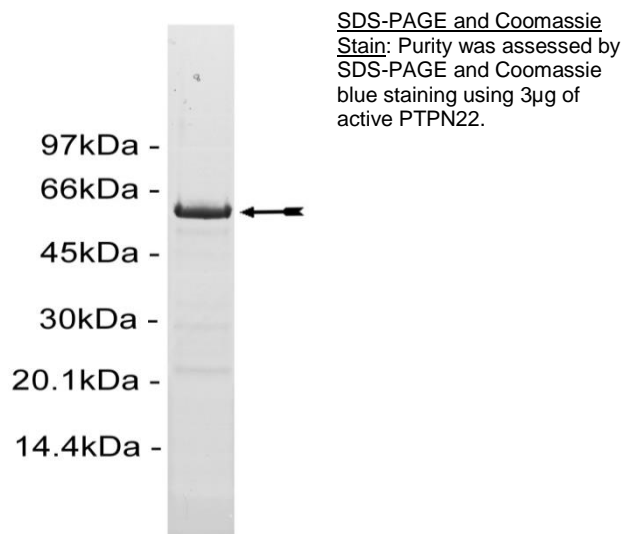
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 0.18–1.4ng of this lot of enzyme dephosphorylated 200µM DiFMUP in the assay described on page two. Assay background was subtracted from the actual Fluorescence Intensity (FI) to yield the results shown below. Quantification of FI was against a 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) standard curve.



MS Tryptic Fingerprint: Confirmed identity as PTPN22 with the translated sequence listed on page three.



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Phosphatase Assay Protocol

Stock Solutions:

1. **Reaction Buffer:** 32mM Hepes pH7.2, 50mM NaCl, 2.5mM EDTA, 0.17mM DTT, 0.83 (v/v)% glycerol, 0.017 (w/v)% BSA, 0.002% Brij-35.
2. 500 μ M DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500 μ M DiFMU (Molecular Probes Catalogue# D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **PTPN22** in reaction buffer and use 0.4–1.4ng in 15 μ l per assay point.
2. Add 10 μ l DiFMUP 500 μ M stock solution (200 μ M final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5 μ l of 100mM sodium orthovanadate.
5. Read FI using an appropriate reader (Excitation 340nm; Emission 450nm).
6. Subtract the zero enzyme values from each FI reading and calculate the enzyme activity by conversion to nmoles product formed using a DiFMU standard calibration curve.

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PTPN22 Sequence Information

<u>Protein</u>	human PTPN22
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human PTPN22
<u>Accession number</u>	GenBank NM_015967

Recombinant PTPN22 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAI PQID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LVPRGSPEFM  DQREILQKFL
241  DEAQSKKITK  EEFANEFLKL  KRQSTKYKAD  KTYPTTVAEK  PKNIKKNRYK  DILPYDYSRV
301  ELSLITSD ED  SSYINANFIK  GVYGPKAYIA  TQGPLSTLL  DFWRMIWEYS  VLIIVMACME
361  YEMGKKK CER  YWAEPGEMQL  EFGPFVS SCE  AEKRKSDYII  RTLKVKFNSE  TRTIYQFHYK
421  NWPDHDV PSS  IDPILELIWD  VRCYQEDDSV  PICIHCSAGC  GRTGVICAID  YTWMLLKDGI
481  IPENFSV FSL  IREMRTQRPS  LVQTQEYEL  VYNAVLELFK  RQMDVIRDKH  SGTESQAKHC
541  I

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Recombinant PTPN22 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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1441  attcctgaga  acttcagtgt  tttcagtttg  atccgggaaa  tgccgacaca  gaggccttca
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1561 agacagatgg atgttatcag agataaacat tctggaacag agagtcaagc aaagcattgt  
1621 atttaa
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