

Certificate of Analysis

SHP-1, active

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

Item # 14-591, 14-591-K, 14-591M

Parent Lot # 1638881

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

Product Description: N-terminal GST-tagged, recombinant, human SHP-1, full length, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 63.2% by SDS-PAGE and Coomassie blue staining. MW = 95kDa.

Specific Activity (Parent lot# 1638881): 1235U/mg, where one unit of SHP-1 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: 0.494mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10% glycerol, 0.03% Brij-35, 0.1mM EGTA, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

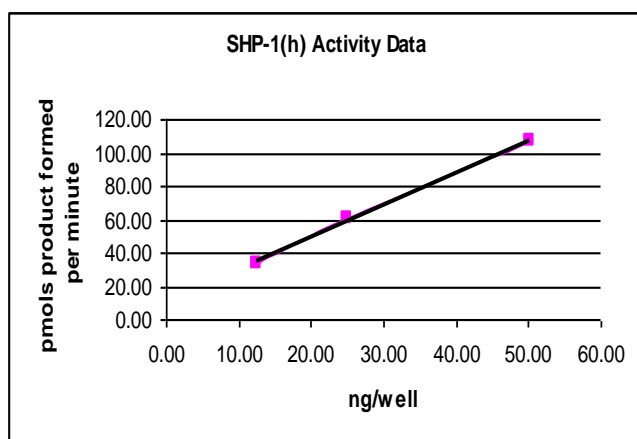
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

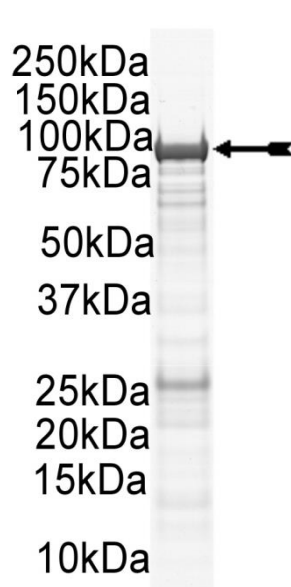
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 12–50ng 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 product identity as SHP-1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active SHP-1.

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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 **SHP-1** in reaction buffer and use 12–50ng 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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SHP-1 Sequence Information

<u>Protein</u>	SHP-1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M237 of the recombinant protein is equivalent to M1 of human SHP-1
<u>Accession number</u>	GenBank NM_002831

Recombinant SHP-1 amino acid sequence:

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1  MSPIILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPGIP  SPEAPRMVRW
241  FHRDLSGLDA  ETLKGRGVH  GSFLARPSRK  NQGDFLSLVR  VGDQVTHIRI  QNSGDFYDLY
301  GGEKFATLTE  LVEYYTQQQG  VLQDRDGTII  HLKYPLNCSD  PTSERWYHGH  MSGGQAETLL
361  QAKGEPWTF  VRESLSQPGD  FVLSVLSQDP  KAGPGSPLRV  THIKVMCEGG  RYTVGGLETF
421  DSLTDLVEHF  KKTGIEEASG  AFVYLRQPY  ATRVNAADIE  NRVLELNKKQ  ESED TAKAGF
481  WEEFESLQKQ  EVKNLHQRLE  GORPENKGN  RYKNILPFDH  SRVILQGRDS  NIPGSDYINA
541  NYIKNQLLGP  DENAKTYIAS  QGCLEATVND  FWQMAWQENS  RVIVMTTREV  EKGRNKCPY
601  WPEVGMQRAY  GPYSVTNCGE  HDTTEYKLR  LQVSPLDNGD  LIREIWHYQY  LSWPDHGVPS
661  EPGGVL SFLD  QINQRQESLP  HAGPIIVHCS  AGIGRTGTII  VIDMLMENIS  TKGLDCDIDI
721  QKTIQMVRAQ  RSGMVQTEAQ  YKFIYVAIAQ  FIETTKKLE  VLQSQKQES  EYGNITYPPA
781  MKNAHAKASR  TSSKHKEDVY  ENLHTKNKRE  EKVKKQRSAD  KEKSKGSLKR  K

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Recombinant SHP-1 nucleotide sequence:

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2461 aaggagaaga gcaagggttc cctcaagagg aagtga
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