

Certificate of Analysis

SHP-2, active

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

Item # 14-622, 14-622-K, 14-622M

Parent Lot # 196949

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 SHP-2, amino acids 230–545, expressed in *E. coli* cells. Purified using glutathione agarose.

Purity 76% by SDS-PAGE and Coomassie blue staining. MW = 63.7kDa.

Specific Activity (Parent lot# 196949): 14211U/mg, where one unit of SHP-2, active 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: 1.93mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10% glycerol, 0.1mM EGTA, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol Frozen solution.

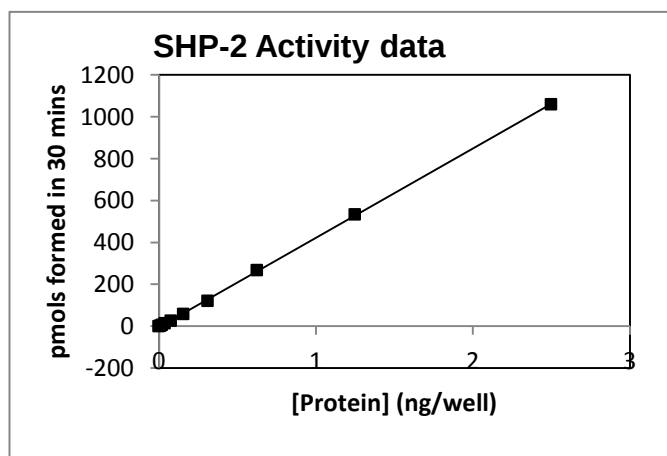
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

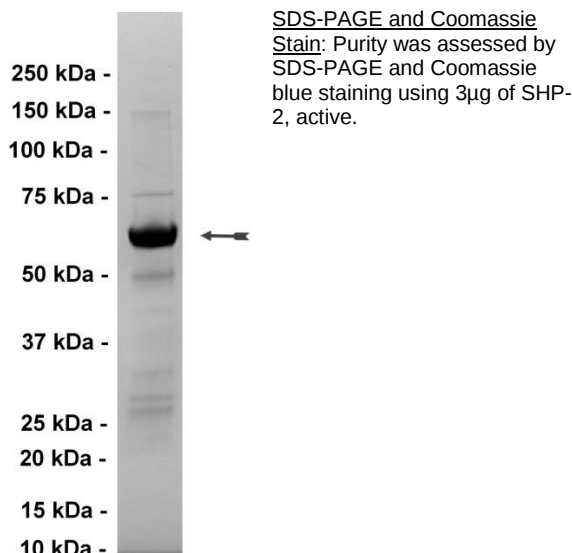
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 0.01–2.5ng 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 SHP-2 with the translated sequence listed on page three.



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Phosphatase Assay Protocol

Stock Solutions:

1. **Reaction Buffer:** 60mM Hepes pH7.2, 150mM NaCl, 1mM EDTA, 0.17mM DTT, 0.83 (v/v)% glycerol, 0.017 (w/v)% BSA, 0.002% Brij-35.
2. 500µM DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500µM DiFMU (Molecular Probes Catalogue# D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **SHP-2** in reaction buffer and use 0.01–2.5ng in 15µl per assay point.
2. Add 10µl DiFMUP 500µM stock solution (200µM final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5µl 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-2 Sequence Information

<u>Protein</u>	Human SHP-2
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	V230 of recombinant sequence is equivalent to V230 of native human SHP-2
<u>Accession number</u>	GenBank X70766

Recombinant SHP-2 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCCL  DAFPKLVCFK
181  KRIEAIPIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPEFV  RELSKLAETT
241  DKVKQGFWEF  FETLQQQECK  LLYSRKEGQR  QENKNKNRYK  NILPFDHTRV  VLHDGDPNEP
301  VSDYINANII  MPEFETKCNN  SKPKKSYIAT  QGCLQNTVND  FWRMVFQENS  RVIVMTTKEV
361  ERGKSKCVKY  WPDEYALKEY  GVMRVRNVKE  SAAHDYTLRE  LKLSKVGQGN  TERTVWQYHF
421  RTWPDHGVPS  DPGGVLDGLE  EVHHKQESIM  DAGPVVVHCS  AGIGRTGTFI  VIDILIDIIR
481  EKGVDCDIDV  PKTIQMVRSQ  RSGMVQTEAQ  YRFIYMAVQH  YIETLQRRIE  EEQSKSRKGH
541  EYTNI

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Recombinant SHP-2 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacgtt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatatta  atggtgatca  tgtaacccat  cctgacttca  tgttgatatga  cgctcttgat
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1201  cttaaacttt  caaaggttgg  acaagggaat  acggagagaa  cggctctggc  ataccacttt
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1441  gagaaagggt  ttgactgcga  tattgacgtt  cccaaaacca  tccagatggt  gcggtctcag
1501  aggtcaggga  tgggtccagac  agaagcacag  taccgattta  tctatatggc  ggtccagcat

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1561 tatattgaaa cactacagcg caggattgaa gaagagcaga aaagcaagag gaaagggcac
1621 gaatatacaa atatttga

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