

Certificate of Analysis

SRPK1, active

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

Item # 14-564, 14-564-K, 14-564M

Parent Lot # 1640107

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 SRPK1, amino acids 2–end. Expressed in *E. coli* cells. Purified using glutathione agarose. Purity 64.1% by SDS-PAGE and Coomassie blue staining. MW = 101kDa.

Specific Activity (Parent lot# 1640107): 4084U/mg, where one unit of SRPK1, active activity is defined as 1nmol phosphate incorporated into 300μM (RSRSRSRSRSRSRSR) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.417mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

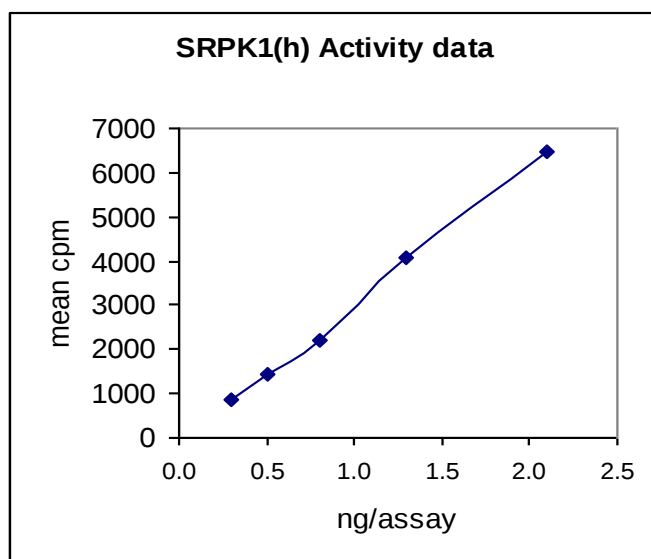
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

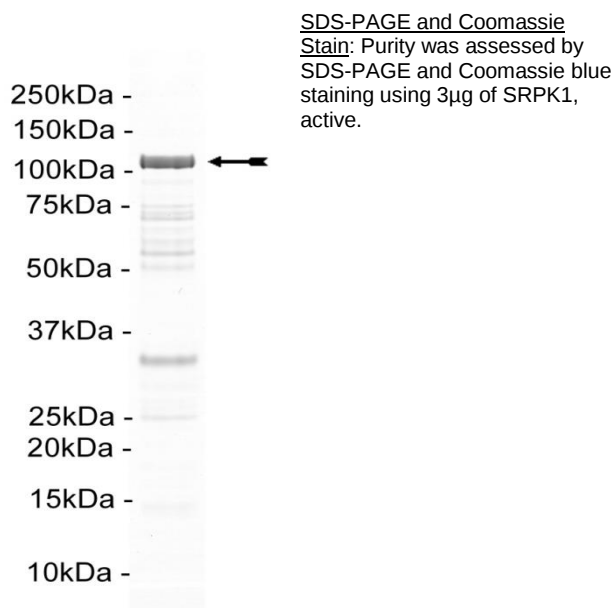
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.3–2.1ng of this lot of enzyme phosphorylated 300μM (RSRSRSRSRSRSRSR) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as SRPK1 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(RSRSRSRSRSRSRSR):** Use at a final concentration of 300 μ M. Make a 3mM stock. Add 2.5 μ l of stock per assay point.
3. **SRPK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.3–2.1ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(RSRSRSRSRSRSRSR)**.
3. Add **2.5 μ l (0.3–2.1ng) SRPK1, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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SRPK1 Sequence Information

<u>Protein</u>	Human SRPK1
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	E232 of recombinant sequence is equivalent to E2 of native human SRPK1
<u>Accession number</u>	GenBank NM_003137

Recombinant SRPK1 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPLG  SERKVLALQA
241  RKKRTKAKKD  KAQRKSETQH  RGSAPHSESD  LPEQEEIILG  SDDDEQEDPN  DYCKGGYHLV
301  KIGDLFNTRY  HVIRKLWGH  FSTVLSWDI  QGKKFVAMKV  VKSAEHTTET  ALDEIRLLKS
361  VRNSDPNDPN  REMVVQLLDD  FKISGVNGTH  ICMVFEVLGH  HLLKWIISN  YQGLPLPCVK
421  KIIQQVLQGL  DYLHTKCRII  HTDIKPENIL  LSVNEQYIRR  LAEEATEWQR  SGAPPPSGSA
481  VSTAPQPKPA  DKMSKNKKKK  LKKKQKRQAE  LLEKRMQEIE  EMEKESGPGQ  KRPNKQEESE
541  SPVERPLKEN  PPNKMTQEK  EESSTIGQDQ  TLMERDTEGG  AAEINCNGVI  EVINYTQNSN
601  NETLRHKEDL  HNANDCDVQN  LNQESSFLSS  QNGDSSTSQE  TDSTPITSE  VSDTMVCQSS
661  STVGQSFSEQ  HISQLQESIR  AEIPCEDEQE  QEHNGPLDNK  GKSTAGNFLV  NPLEPKNAEK
721  LKVKIADLGN  ACWVHKHFT  DIQTRQYRSL  EVLIGSGYNT  PADIWSTACM  AFELATGDYL
781  FEPHSGEYYT  RDEDHIALII  ELLGKVPKRL  IVAGKYSKEF  FTKKGLDKHI  TKLKPWGLFE
841  VLVEKYEWSQ  EEAAGFTDFL  LPMLELIPEK  RATAAECLRH  PWLNS

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Recombinant SRPK1 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatt  ttgaagaaaa  atatgaagag  catttgtatg  agcgcatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgaacccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
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1321  cacactgaca  ttaaaccaga  gaacatctta  ttgtcagtga  atgagcagta  cattcggagg
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1501  ttgaagaaga  agcagaagcg  ccaggcagaa  ttactagaga  agcgaatgca  ggaaattgag
1561  gaaatcgaga  aagatcgagg  ccctgggcaa  aaaagaccaa  acaagcagga  agaatacag
1621  agtcctgttg  aaagaccctt  gaaagagaac  ccacctaata  aaatgaccca  agaaaaactt

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1681 gaagagtcaa gtaccattgg ccaggatcaa acgcttatgg aacgtgatac agaggggtggt
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1801 aatgaaacat tgagacataa agaggatcta cataatgcta atgactgtga tgtccaaaat
1861 ttgaatcagg aatctagttt cctaagctcc caaaatggag acagcagcac atctcaagaa
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2221 gatattcaaa caaggcaata tcgttccttg gaagttctaa tcggatctgg ctataatacc
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2581 ctgcccattg tggagctgat ccctgagaag agagccactg ccgccgagtg tctccggcac
2641 ccttggtcta actcctaa

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