

Certificate of Analysis

SRPK2, active

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

Item # 14-666, 14-666-K, 14-666M

Parent Lot # 1608776

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 SRPK2, amino acids 46–end. Expressed in *E. coli* cells. Purified using glutathione agarose. Purity 66.3% by SDS-PAGE and Coomassie blue staining. MW = 100kDa.

Specific Activity (Parent lot# 1608776): 5280U/mg, where one unit of SRPK2, active activity is defined as 1nmol phosphate incorporated into 300µM (RSRSRSRSRSRSR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.182mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

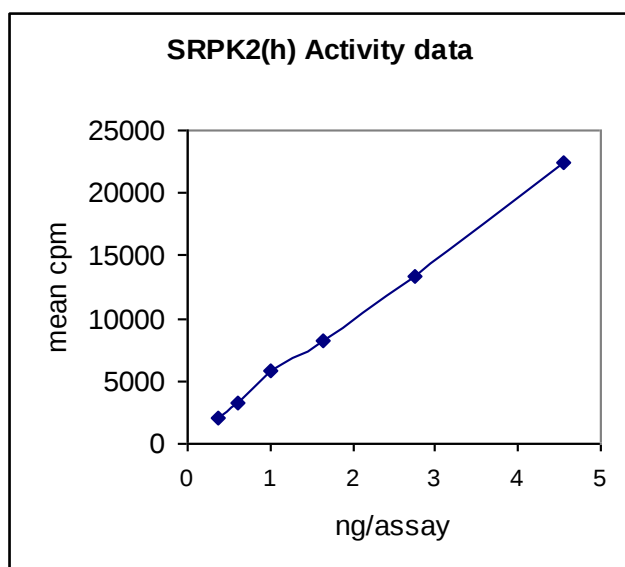
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

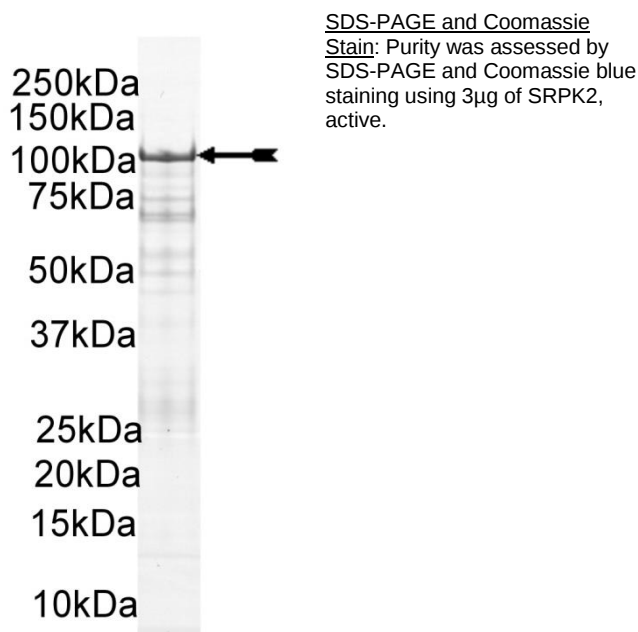
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.4–4.6ng of this lot of enzyme phosphorylated 300µM (RSRSRSRSRSRSR) 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 SRPK2 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. **SRPK2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.4–4.6ng 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:

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 (0.4–4.6ng) SRPK2, 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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SRPK2 Sequence Information

Protein	Human SRPK2
Tags	N-terminal GST
Native sequence	E234 of the recombinant protein is equivalent to E46 of human SRPK2
Accession number	GenBank NM_182691

Recombinant SRPK2 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 VVLYMDPMCL DAFPKLVCFK
181 KRTEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFP GRQEEIILGS
241 DDEEQEDPAD YCKGGYHPVK IGDLFNGRYH VIRKLGWGHF STVWLCWDMQ GKRFFVAMKV
301 KSAQHYTETA LDEIKLLKCV RESDPSDPNK DMVVQLIDDF KISGMNGIHV CMVFEVLGHH
361 LLKWIISNY QGLPVRCVKS IIRQVLQGLD YLHCKCKIIH TDIKPENILM CVDDAYVRRM
421 AAEATEWQKA GAPPPSGSAV STAPQQKPIG KISKNNKKKL KKKQKRQAEI LEKRLQEIEE
481 LEREAERKII EENITSAAPS NDQDGEYCPE VKLKTGLEE AAEAETAKDN GEAEDQEEKE
541 DAEKENIEKD EDDVDQELAN IDPTWIESPK TNGHIENGPF SLEQQLDDED DDEEDCPNPE
601 EYNLDEPNAE SDYTYSSSYE QFNGELPNGR HKIPESQFPE FSTSLFSGSL EPVACGSVLS
661 EGSPLTEQEE SSPSHDRSRT VSASSTGDL PAKTRAADLL VNPLDPRNAD KIRVKIADLG
721 NACVWHKHFT EDIQRQYRS IEVLIGAGYS TPADIWSTAC MAFELATGDY LFEPHSGEDY
781 SRDEDHIAHI IELLSIPRH FALSGKYSRE FFNRRGELRH ITKLKPWSLF DVLVEKYGWP
841 HEDAAQFTDF LIPMLEMVPE KRASAGECLR HPWLNS

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Recombinant SRPK2 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
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121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgtat
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1501 aatgaccagg atggcgaata ctgcccagag gtgaaactaa aaacaacagg attagaggag
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1621 gatgctgaga aagaaaacat tgaaaaagat gaagatgatg tagatcagga acttgcgaa
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1861 caattcaatg gtgaattgcc aaatggacga cataaaattc ccgagtcaca gttcccagag
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2581 aaacgagcct cagctggcga atgccttcgg catccttggt tgaattctta g

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Reviewed and approved by site quality representative.

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