

Certificate of Analysis

STK39, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-049, 16-049-K, 16-049M

Parent Lot # D18HP003N

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 STK39 full length and N-terminal GST tagged, recombinant, human MO25 α full length, co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Complex activated *in vivo* with recombinant WNK3. Purity 97% (combined) by SDS-PAGE and Coomassie blue staining. STK39 MW = 91kDa MO25 α MW = 67kDa.

Specific Activity (Parent lot# D18HP003N): 132U/mg, where one unit of STK39 activity is defined as 1nmol phosphate incorporated into 125 μ M catchtide per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.70mg/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.

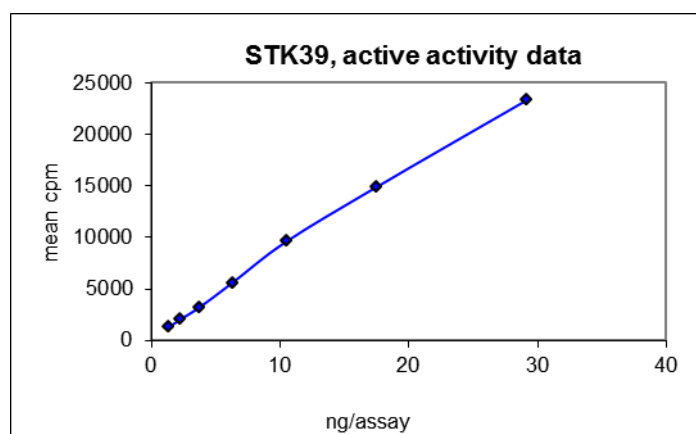
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

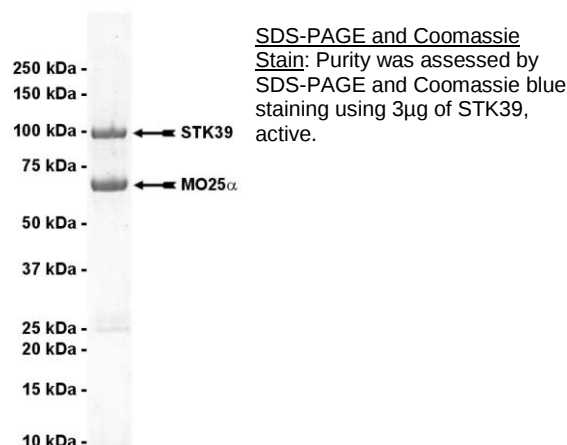
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.36–29.17ng of this lot of enzyme phosphorylated 125 μ M catchtide 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 identity as STK39 and MO25 α with the translated sequences listed on pages three and five.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Catchtide:** Use at a final assay concentration of 125µM. Prepare a 2.5mM stock and add 1.25µl of stock per assay point.
- 3. STK39, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.36–29.17ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 1.25µl of catchtide.
3. Add **2.5µl (1.36-29.17ng) STK39, active.**
4. Add 6.25µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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STK39, active Sequence Information

<u>Protein</u>	Human STK39
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M241 of the recombinant protein is equivalent to M1 of human STK39.
<u>Accession number</u>	GenBank NM_013233.1

Recombinant STK39 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 KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFK GLRRRIQIFH
241 MAEPSGSPVH VQLPQQAAPV TAAAAAAPAA ATAAPAPAAP AAPAPAPAPA PAAQAVGWPI
301 CRDAYELQEV IGSAGATAVVQ AALCKPRQER VAIKRINLEK CQTSMDLLK EIQAMSQCSH
361 PNVVTTYTSF VVKDELWLVM KLLSGGSMLD IIKYIVNRGE HKNGVLEEAI IATILKEVLE
421 GLDYLHRNGQ IHRDLKAGNI LLGEDGSVQI ADFGVSAFLA TGGDVTRNKV RKTFTVGTCPW
481 MAPEVMEQVR GYDFKADMWS FGITAIELAT GAAPYHKYPP MKVLMLTLQN DPPTLETGVE
541 DKEMMKKYGK SFRKLLSLCL QKDPSKRPTA AELLKCKFFQ KAKNREYLIE KLLTRTPDIA
601 QRAKKVRRVP GSSGHLHKTE DGDWEWSDE MDEKSEEGKA AFSQEKSRV KEENPEIAVS
661 ASTIPEQIQS LSVHDSQGP NANEDYREAS SCAVNLVLR LNSRKELNDI RFEFTPGRDT
721 ADGVSQELFS AGLVDGHDV IVAANLQKIV DDPKALKTLT FKLASGCDGS EIPDEVKLIG
781 FAQLSVSLEK KSRILDPIRN ASAACVPSL SRSTRGS

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Recombinant STK39 nucleotide sequence:

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1  atgtccccta tactagggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatga agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
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241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatattaa atggtgatca tgtaacccat cctgacttca tgttgatga cgtcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
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721 atggcgggagc cgagcggctc gcccggtcac gtccagcttc cccagcagge ggcggcggtg
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1261 ggcttagact atctacacag aaacggtcag attcacaggg atttgaaagc tggtaatatt
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1381 acaggggggtg atgttaccgc aaataaagta agaaaaacat tcgttggcac cccatgttgg
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1681 cagaaagatc cttccaaaag gccacagca gcagaacttt taaaatgcaa attcttccag
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1861 gacggggact gggagtggag tgacgacgag atggatgaga agagcgaaga agggaaagca
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2161 gcagatgggt tatctcagga gctcttctct gctggcttgg tggatgggtca cgatgtagtt
2221 atagtggctg ctaatttaca gaagattgta gatgatccca aagctttaaa aacattgaca
2281 ttttaagttg cttctggctg tgatgggtcg gagattcctg atgaagtga gctgattggg
2341 tttgctcagt tgagtgtcag cctcgagaaa aaatcacgaa ttctggatcc gatacgtaac
2401 gcgtctgcag catgcgtggg accaagcttg tcgagaagta ctagaggatc ataa

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MO25α Sequence Information

Protein	Human MO25α
Tags	N-terminal GST
Native sequence	M232 of the recombinant protein is equivalent to M1 of human MO25α
Accession number	GenBank NM_016289.2

Recombinant MO25α 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  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPLG  SMPFFPGKSH
241  KSPADIVKNL  KESMAVLEKQ  DISDKKAeka  TEEVSKNLVA  MKEILYGTNE  KEPQTEAVAQ
301  LAQELYNSGL  LSTLVADLQL  IDFEGKKDVA  QIFNNILRRQ  IGTTRPTVEY  ICTQQNILFM
361  LLKGYESPEI  ALNCGIMLRE  CIRHEPLAKI  ILWSEQFYDF  FRYVEMSTFD  IASDAFATFK
421  DLLTRHKLLS  AEFLEQHYDR  FFSEYEKLLH  SENYVTKRQS  LKLLGELLLD  RHNFTIMTKY
481  ISKPENLKLM  MNLLRDKSRN  IQFEAFHVFK  VFVANPNKTQ  PILDILLKNQ  AKLIEFLSKF
541  QNDRTEDEQF  NDEKTYLVKQ  IRDLKRPAQQ  EA

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Recombinant MO25α nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaataat  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
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1621  cagaacgaca  ggacggagga  tgagcagttt  aacgacgaga  agacctattt  agttaaacag
1681  atcagggatt  tgaagagacc  agctcagcaa  gaagcttaa
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