

Certificate of Analysis

SIK3, active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-011, 15-011-K, 15-011M

Parent Lot # 215042

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 and C-terminal 6His-tagged, recombinant, human SIK3 amino acids 1-307 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 76% by SDS-PAGE and Coomassie blue staining. MW = 63 kDa.

Specific Activity (Parent lot# 215042): 444 U/mg, where one unit of SIK3 activity is defined as 1 nmol phosphate incorporated into 100 μ M CHKtide (KKKVSRSGLYRSPSPENLNRPR) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.37 mg/ml of enzyme in 25 mM Tris/HCl pH 7.5, 300 mM NaCl, 0.05 mM EGTA, 50% (v/v) glycerol, 75 mM imidazole, 0.5 mM benzamidine, 0.1 mM PMSF, 0.05% 2-mercaptoethanol. Frozen solution.

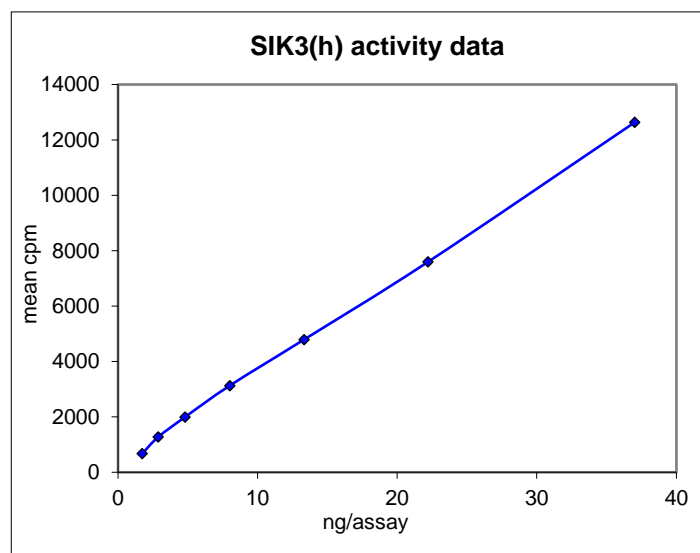
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

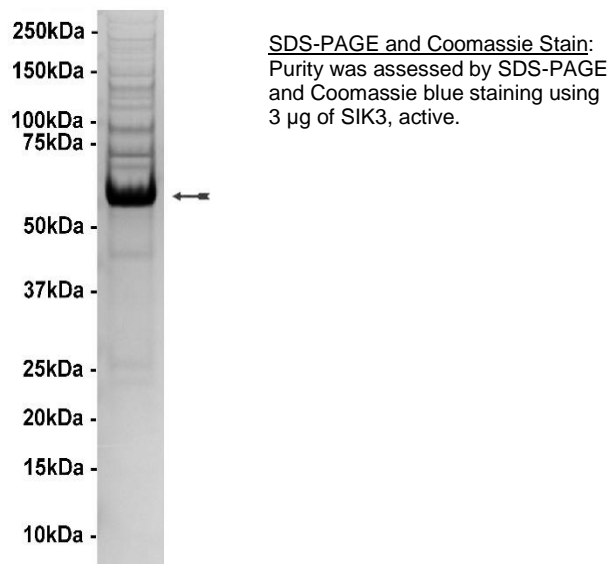
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.7–37.0 ng of this lot of enzyme phosphorylated 100 μ M CHKtide 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 SIK3 with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
- 2. CHKtide (KKKVSRSGLYRSPSPENLNRPR):** Use at a final assay concentration of 100 μ M. Prepare a 1 mM stock and add 2.5 μ l of stock per assay point.
- 3. SIK3, active:** Dilute with 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 1.7–37.0 ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 – 800 cpm/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 CHKtide (KKKVSRSGLYRSPSPENLNRPR).
3. Add **2.5 μ l (1.7–37.0 ng) SIK3, 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 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the dried filtermat to a sealable plastic bag and add 4 ml 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.

Certificate of Analysis

SIK3, active Sequence Information

Protein	Human SIK3
Tags	N-terminal GST and C-terminal 6His
Native sequence	M230 of the recombinant protein is equivalent to M1 of human SIK3
Accession number	GenBank BC128510.1

Recombinant SIK3 amino acid sequence:

```

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  LVPRGSKEFM  PARIGYYEID
241  RTIGKGNFAV  VKRATHLVTK  AKVAIKIIDK  TQLDEENLKK  IFREVQIMKM  LCHPHIIRLY
301  QVMETERMIY  LVTEYASGGE  IFDHLVAHGR  MAEKEARRKF  KQIVTAVYFC  HCRNIVHRDL
361  KAENLLLDAN  LNIKIADFGF  SNLFTPGQLL  KTWCGSPPYA  APELFEGKEY  DGPVKDIWSL
421  GVVLYVLVCG  ALPFDGSTLQ  NLRARVLSGK  FRIPFFMSTE  CEHLIRHMLV  LDPNKRLSME
481  QICKHKWMKL  GDADPNFDRL  IAECQQLKEE  RQVDPLNEDV  LLAMEDMGLD  KEQTLQGGPG
541  GHHHHHH

```

Recombinant SIK3 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
181  ggtgatgta  aattaacaca  gtctatggcc  atcatacgtt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggttccgc  gtggatccaa  ggaattcatg  cccgcccgta  tcggctacta  cgagatcgac
721  cgcaccatcg  gcaagggcaa  cttcgcggtg  gtcaagcggg  ccacgcacct  cgtcaccaag
781  gccaaagttg  ctatcaagat  catagataag  acccagctgg  atgaagaaaa  cttgaagaag
841  attttccggg  aagttcaaat  tatgaagatg  ctttgccacc  cccatatcat  caggctctac
901  caggttatgg  agacagaacg  gatgatttat  ctggtgacag  aatatgctag  tggaggggaa
961  atatttgacc  acctggtggc  ccatggtaga  atggcagaaa  aggaggcacg  tcggaagttc
1021  aaacagatcg  tcacagctgt  ctatttttgt  cactgtcgga  acattgttca  tcgtgattta
1081  aaagctgaaa  atttacttct  ggatgccaat  ctgaatatca  aaatagcaga  ttttggtttc
1141  agtaacctct  tcaactctgg  gcagctgctg  aagacctggt  gtggcagccc  tcctatgct
1201  gcacctgaac  tctttgaagg  aaaagaatat  gatgggccc  aagtggacat  ctggagcctt
1261  ggagttgtcc  tctacgtgct  tgtgtgcggt  gccctgccat  ttgatggaag  cacactgcag
1321  aatctgctgg  cccgcgtgct  gagtggaaag  ttccgcatcc  cattttttat  tccacagaa
1381  tgtgagcatt  tgatccgcca  tatgttggtg  ttagatccca  ataagcgcct  ctccatggag
1441  cagatctgca  agcacaagtg  gatgaagcta  ggggacgccc  atcccactt  tgacagggta
1501  atagctgaat  gccacaact  aaaggaagaa  agacaggtgg  acccctgaa  tgaggatgtc
1561  ctcttgccca  tggaggacat  gggactggac  aaagaacaga  cactgcaggg  cccgggccc
1621  ggccatcacc  atccatcata  ctaa

```

Certificate of Analysis

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.