

Certificate of Analysis

SIK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-010, 15-010-K, 15-010M

Parent Lot # 209024

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 SIK2 amino acids 1-276 expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 76% by SDS-PAGE and Coomassie blue staining. MW = 59 kDa.

Specific Activity (Parent lot# 209024): 370 U/mg, where one unit of SIK2 activity is defined as 1 nmol phosphate incorporated into 100 μ M KKKVSRSGLYRSPSPENLNRPR per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 1.98 mg/ml of enzyme in 50 mM Tris/HCl pH7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

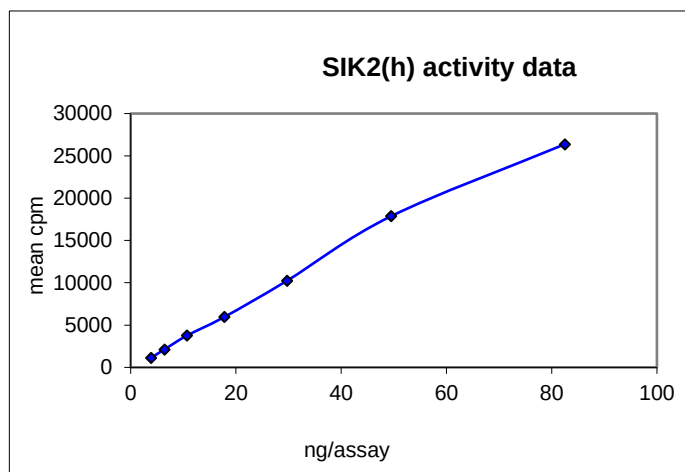
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

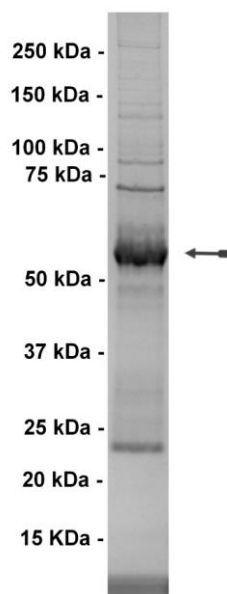
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 3.9–82.5 ng of this lot of enzyme phosphorylated 100 μ M KKKVSRSGLYRSPSPENLNRPR 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 SIK2 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of SIK2, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH7.0, 1 mM EDTA.
- 2. 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. SIK2, active:** Dilute with 20 mM MOPS/NaOH pH7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 3.9–82.5 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 5.0 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of KKKVSRSGLYRSPSPENLNRPR.
3. Add **2.5 μ l (3.9–82.5 ng) SIK2, active.**
4. Add 5.0 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 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.

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SIK2, active Sequence Information

Protein	Human SIK2
Tags	N-terminal GST
Native sequence	M230 of the recombinant protein is equivalent to M1of human SIK2
Accession number	GenBank NM_015191.1

Recombinant SIK2 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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFM VMADGPRHLQ
241 RGPVRVGFYD IEGTLGKGNF AVVKLGRHRI TKTEVAIKII DKSQLDVNL EKIYREVQIM
301 KMLDHPHIIK LYQVMETKSM LYLVTYAKN GEIFDYLANH GRLNESEARR KFWQILSAVD
361 YCHGRKIVHR DLKAENLLLD NNMNIKIADF GFGNFFKSGE LLATWCGSPP YAAPEVFEGQ
421 QYEGPQLDIW SMGVVLYVLV CGALPFDGPT LPILRQVLE GRFRIPYFMS EDCEHLIRRM
481 LVLDPSKRLT IAQIKEHKWM LIEVP
  
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Recombinant SIK2 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatc ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatgat
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1501 ctcatagaag ttccttga
  
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Reviewed and approved by site quality representative.

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