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Certificate of Analysis

SIK, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-652, 14-652-K, 14-652M
Parent Lot # D7BN050U

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 6His-tagged, recombinant, human SIK, amino acids 1–281, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 52% by SDS-PAGE and Coomassie blue staining. MW = 36.2kDa.

Specific Activity (Parent lot# D7BN050U): 451U/mg, where one unit of SIK, active activity is defined as 1nmol phosphate incorporated into 100µM (AMARAASAAALARRR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.918mg/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.

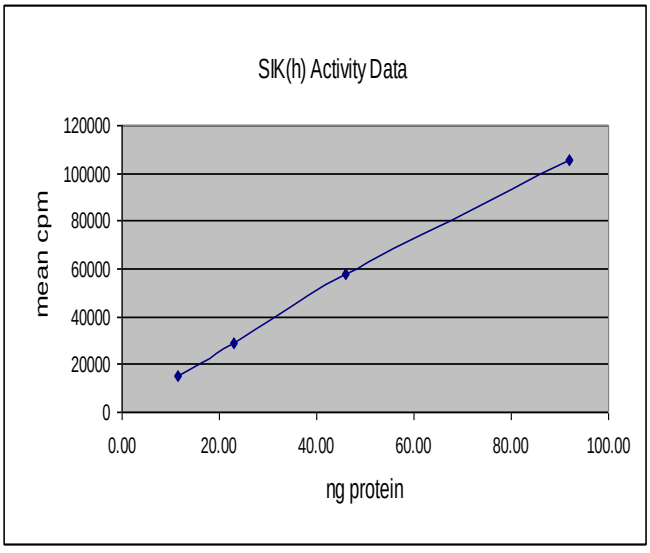
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

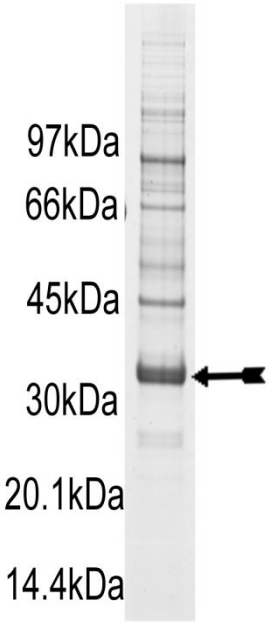
Quality Control Testing

Kinase Assay: 11–92ng of this lot of enzyme phosphorylated 100µM (AMARAASAAALARRR) 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 SIK with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of SIK, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(AMARAASAAALARRR):** Use at a final concentration of 100µM. Make up a 1mM stock. Add 2.5µl of stock per assay point.
3. **SIK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11–92ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 2.5µl of **(AMARAASAAALARRR)**.
3. Add **2.5µl (11–92ng) SIK, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 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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SIK Sequence Information

Protein Human SIK

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M1 of human SIK

Accession number Genbank NM_173354

Recombinant SIK amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MVIMSEFSAD PAGQGQGQQK PLRVGFYDIE
 61 RTLGKGNFAV VKLARHRVTK TQVAIKIIDK TRLDSSNLEK IYREVQLMKL LNHPHIIKLY
121 QVMETKDMLY IVTEFAKNGE MFDYLTSNGH LSENEARKKF WQILSAVEYC HDHHIVHRDL
181 KTENLLLDGN MDIKLADFGF GNFYKSGEPL STWCGSPPYA APEVFEGKEY EGPQLDIWSL
241 GVVLYVLVCG SLPFDGPNLP TLRQRVLEGR FRIPFFMSQD CESLIRRMLV VDPARRITIA
301 QIRQHRWMRA E
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Recombinant SIK nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgga tccggaattc atggttatca tgtcggagtt cagcgcggac
121 cccgcgggcc agggtcaggg ccagcagaag cccctccggg tgggttttta cgacatcgag
181 cggaccctgg gcaaaggcaa cttcgcggtg gtgaagctgg cgcggcatcg agtcaccaaa
241 acgcaggttg caataaaaat aattgataaa acacgattag attcaagcaa tttggagaaa
301 atctatcgtg aggttcagct gatgaagctt ctgaaccatc cacacatcat aaagctttac
361 caggttatgg aaacaaagga catgctttac atcgtcactg aatttgctaa aaatggagaa
421 atgtttgatt atttgacttc caacgggcac ctgagtgaga acgaggcgcg gaagaagttc
481 tggcaaatcc tgtcggccgt ggagtactgt cacgaccatc acatcgtcca ccgggacctc
541 aagaccgaga acctcctgct ggatggcaac atggacatca agctggcaga ttttggattt
601 gggaatttct acaagtcagg agagcctctg tccacgtggt gtgggagccc cccgtatgcc
661 gccccggaag tctttgaggg gaaggagtat gaaggccccc agctggacat ctggagcctg
721 ggcgtggtgc tgtacgtcct ggtctgcggt tctctccccc tcgatgggcc taacctgccg
781 acgctgagac agcgggtgct ggagggccgc ttccgcatcc ccttcttcat gtctcaagac
841 tgtgagagcc tgatccgccg catgctggtg gtggaccccg ccaggcgcat caccatcgcc
901 cagatccggc agcaccggtg gatgcgggct gagtaa
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