

Certificate of Analysis

SNRK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-932, 14-932-K, 14-932M

Parent Lot # D13DP008N

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: C-terminal 6His-tagged, recombinant, human SNRK amino acids 1-505 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography

Purity 91% by SDS-PAGE and Coomassie blue staining. MW = 58kDa.

Specific Activity (Parent lot# D13DP008N): 210U/mg, where one unit of SNRK, active activity is defined as 1nmol phosphate incorporated into 250µM (KKLRRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.231mg/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.

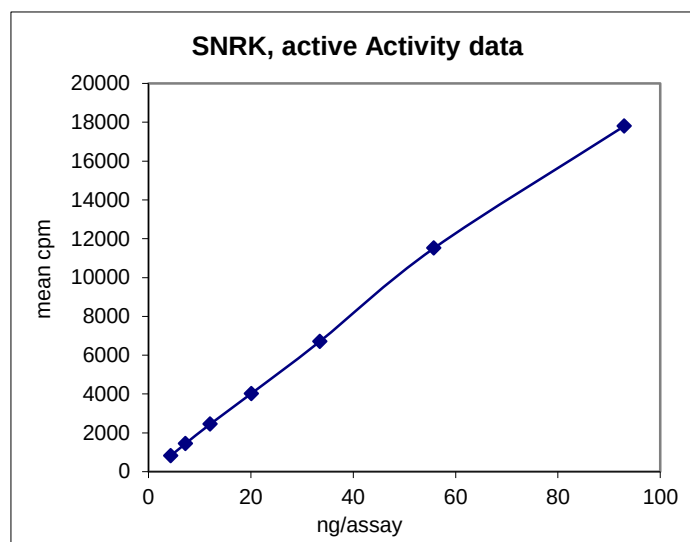
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

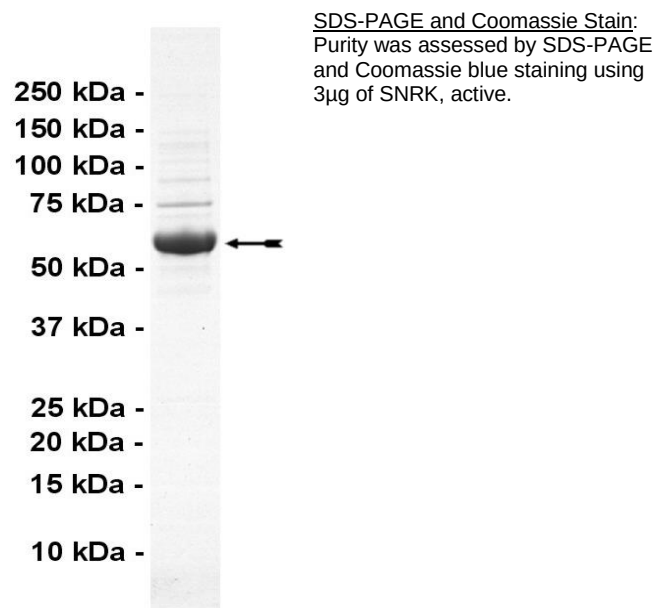
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4–93ng of this lot of enzyme phosphorylated 250µM (KKLRRTLSFAEPG) 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 SNRK with the translated 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. **(KKLRRTLSFAEPG):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **SNRK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–93ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKLRRTLSFAEPG)**.
3. Add **2.5 μ l (4–93ng) SNRK, 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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SNRK Sequence Information

Protein	human SNRK
Tags	C-terminal 6His
Native sequence	M1 of the recombinant protein is equivalent to M1 of human SNRK
Accession number	GenBank NM_017719

Recombinant SNRK amino acid sequence:

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1  MAGFKRGYDG  KIAGLYDLDK  TLGRGHFAVV  KLARHVFTGE  KVAVKVIDKT  KLDTLATGHL
61  FQEVRCMKLV  QHPNIVRLYE  VIDTQTKLYL  ILELGDGGDM  FDYIMKHEEG  LNEDLAKKYF
121 AQIVHAISYC  HKLHVVRDL  KPENVVFEK  QGLVKLTDFG  FSNKFQPGKK  LTTSCGSLAY
181 SAPEILLGDE  YDAPAVDIWS  LGVILFMLVC  GQPPFQEAND  SETLTMIMDC  KYTVPSHSVSK
241 ECKDLITRML  QRDPKRASL  EEIENHPWLQ  GVDPSPATKY  NIPLVSYKNL  SEEEHNSIIQ
301 RMLVLDIADR  DAIVEALETN  RYNHITATYF  LLAERILREK  QEKEIQTRSA  SPSNIKAQFR
361 QSWPTKIDVP  QDLEDDLAT  PLSHATVPQS  PARAADSVLN  GHRSGKLCDS  AKKDDLPELA
421 GPALSTVPPA  SLKPTASGRK  CLFRVEEDEE  EDEEDKKPMS  LSTQVVLRRK  PSVTNRLTSR
481 KSAPVLNQIF  EEGESDDEFD  MDENLHHHHH  H

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Recombinant SNRK nucleotide sequence:

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1  atggcaggat  ttaagcgagg  gtatgatgga  aagattgctg  gattatatga  tctggataaa
61  accttggtgc  gaggccattt  tgccgtgggt  aaacttgcca  ggcattgctt  tacgggtgaa
121 aaggtggcag  taaaagttat  tgacaagaca  aaactggaca  ctctagctac  tgggtcatctt
181 ttccaggaag  tgagatgcat  gaaactagtg  cagcatccta  acatcgctcg  cttttatgaa
241 gttattgaca  ccagaccaa  actatatctt  attctagaac  ttggggatgg  aggagatatg
301 tttgattata  taatgaaaca  tgaggagggt  cttaatgaag  acttggtcaa  gaagtatttt
361 gctcagatag  ttcattgctt  atcttattgc  cataaactcc  atgtggttca  cagagactta
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1501 atggatgaga  atctgcatca  ccatccacct  cactaa

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Reviewed and approved by site quality representative.

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