

Certificate of Analysis

SGK1 (S422D), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-331, 14-331-K, 14-331M

Parent Lot # 1797670

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 SGK1, amino acids 60–end, containing an S422D mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Activated with PDK1 (cat#. 14-452) and repurified using heparin-Sepharose. Purity 98.1% by SDS-PAGE and Coomassie blue staining. MW = 45.7kDa.

Specific Activity (Parent lot# 1797670): 5120U/mg, where one unit of SGK1 (S422D), active activity is defined as 1nmol phosphate incorporated into 30μM modified crosstide (GRPRTSSFAEGKK) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.587mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

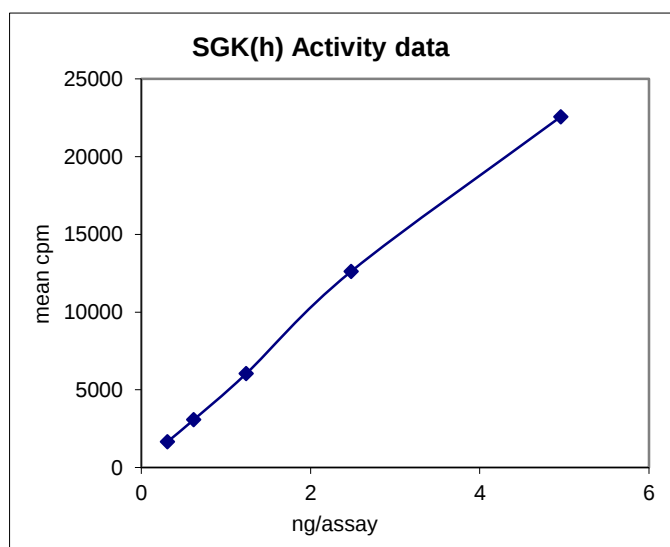
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

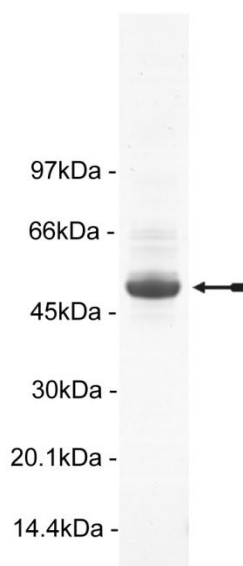
Quality Control Testing

Kinase Assay: 0.3–5.0ng of this lot of enzyme phosphorylated 30μM modified crosstide (GRPRTSSFAEGKK) 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 product identity as SGK1 (S422D) with of 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 SGK1 (S422D), active.



Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Modified Crosstide (GRPRTSSFAEGKK):** Use at a final assay concentration of 30µM. Make a 300µM stock. Add 2.5µl of stock per assay point.
3. **SGK1 (S422D), active:** Dilute with 20mM MOPS/NaOH pH7.0, 0.1mM EDTA, 5% glycerol, 0.01% Bri-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.3–5.0ng per assay point.
4. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[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 2.5µl **modified crosstide**.
3. Add **2.5µl (0.3–5.0ng) SGK1 (S422D), active**.
4. Add 5µl dH₂O.
5. Add 10µl of diluted [γ-³³P]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.

SGK Sequence Information

<u>Protein</u>	Human SGK (S422D)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	I28 of the recombinant protein is equivalent to I60 of human SGK
<u>Accession number</u>	GenBank AF153609. The protein is S422D with respect to this accession number.

Recombinant SGK amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMGISQ PQEPELMNAN PSPPPSPSQQ INLGPSSNP
61 AKPSDFHFLK VIGKGSFGKV LLARHKAEEV FYAVKVLQKK AILKKKEEKH IMSERNVLLK
121 NVKHPFLVGL HFSFQTADKL YFVLDYINGG ELFYHLQRER CFLEPRARFY AAEIASALGY
181 LHSLNIVYRD LKPENILLDS QGHIVLTDFG LCKENIEHNS TTSTFCGTPE YLAPEVLHKQ
241 PYDRTVDWVC LGAVLYEMLY GLPPFYSRNT AEMYDNILNK PLQLKPNITN SARHLLLEGLL
301 QKDRTKRLGA KDDFMEIKSH VFFSLINWDD LINKKITPPF NPNVSGPNDL RHFDPFETEE
361 PVPNSIGKSP DSVLVTASVK EAAEAF LGFD YAPPTDSFL

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Recombinant SGK nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
61 tattttcagg gcgccatggg gatctccaa cctcaggagc ctgagcttat gaatgccaac
121 ccttctcctc caccaagtcc ttctcagcaa atcaaccttg gcccgtcgtc caatcctcat
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