

Certificate of Analysis

SGK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-647, 14-647-K, 14-647M

Parent Lot # 1762413

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 SGK3 amino acids 119–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 79.9% by SDS-PAGE and Coomassie blue staining. MW = 46.7kDa.

Specific Activity (Parent lot# 1762413): 2582U/mg, where one unit of SGK3, active activity is defined as 1nmol phosphate incorporated into Crosstide (GRPRTSSFAEGKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.653mg/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.

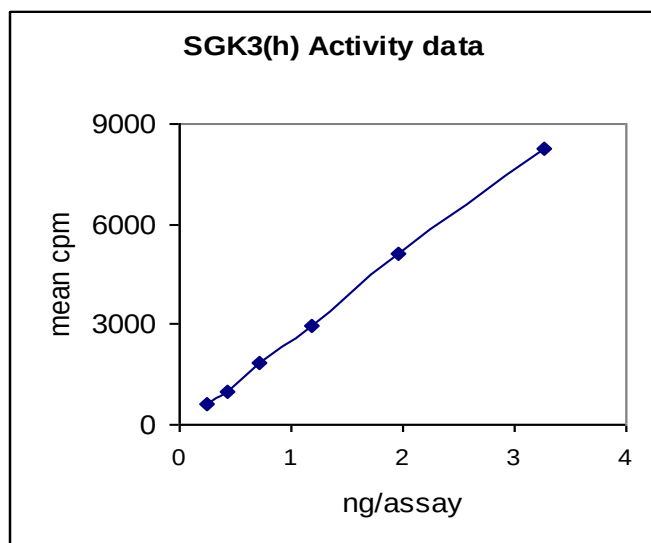
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

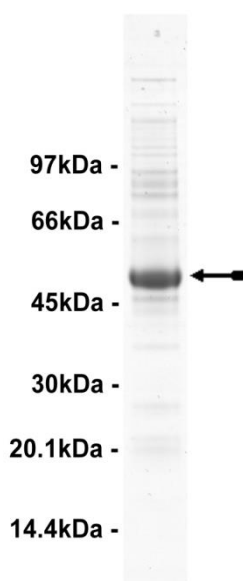
Quality Control Testing

Kinase Assay: 0.3–3.3ng of this lot of enzyme phosphorylated 250µM 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 identity as SGK3 with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of SGK3, active.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Modified Crosstide (GRPRTSSFAEGKK):** Use a final assay concentration of 250 μ M. Make a 2.5mM stock. Add 2.5 μ l of stock per assay point.
- 3. SGK3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.3–3.3ng 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 5 μ l of 5 x reaction buffer per assay to wells
2. Add 2.5 μ l of **modified Crosstide (GRPRTSSFAEGKK)**.
3. Add **2.5 μ l (0.3–3.3ng) SGK3, 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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SGK3 Sequence Information

<u>Protein</u>	human SGK3
<u>Tag</u>	N Terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M119 of human SGK3
<u>Accession number</u>	GenBank NM_013257

Recombinant SGK3 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MDSPKHQSDP SEEDERSSQ KLHSTSQNIN
61 LGPSGNPHAK PTDFDFLKVI GKGSFGKVLL AKRKLDGKFY AVKVLQKKIV LNRKEQKHIM
121 AERNVLLKNV KHPFLVGLHY SFQTTEKLYF VLDFVNGGEL FFHLQRERSF PEHRARFYAA
181 EIASALGYLH SIKIVYRDLK PENILLDSVG HVVLTDFGLC KEGIAISDTT TFCGTPEYL
241 APEVIRKQPY DNTVDWWCLG AVLYEMLYGL PPFYCRDAE MYDNILHKPL SLRPGVSLTA
301 WSILEELLEK DRQNLGAKE DFLEIQNHPP FESLSWADLV QKKIPPPFNP NVAGPDDIRN
361 FDTAFTEETV PYSVCVSSDY SIVNASVLEA DDAFVGFSYA PPSDLFL

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Recombinant SGK3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc atggacagtc caaaacacca gtcagatcca
121 tctgaagatg aggatgaaag aagttctcag aagctacact ctacctcaca gaacatcaac
181 ctgggaccgt ctggaaatcc tcatgccaaa ccaactgact ttgatttctt aaaagttatt
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421 tccttccaaa caactgaaaa gctttatttt gttctggatt ttgttaatgg aggggagctt
481 tttttccact tacaaagaga acggtccttt cctgagcaca gagctagggt ttacgctgct
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721 gcacctgaag taattagaaa acagccctat gacaatactg tagattggtg gtgccttggg
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901 tgggtccattc tggaagaact cctagaaaaa gacaggcaaa atcgacttgg tgccaaggaa
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1021 caaaagaaga ttccaccacc atttaatcct aatgtggctg gaccagatga tatcagaaac
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1141 tctatagtga atgccagtgt attggaggca gatgatgcat tcgttggttt ctcttatgca
1201 cctccttcag aagacttatt tttgtga

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