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Certificate of Analysis

SGK1 (S422D), unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-332, 14-332-K, 14-332M
Parent Lot # 1792535

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 the mutation S422D. Expressed by baculovirus in *Sf*21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 97.3% by SDS-PAGE and Coomassie blue staining. MW = 45.7kDa.

Specific Activity (Parent lot# 1792535): 1451U/mg after activation with PDK1 as described below. One Unit of SGK1 (S422D) activity is defined as 1nmol phosphate incorporated into Crosstide in one minute.

Formulation: 2.935mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months 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 microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C

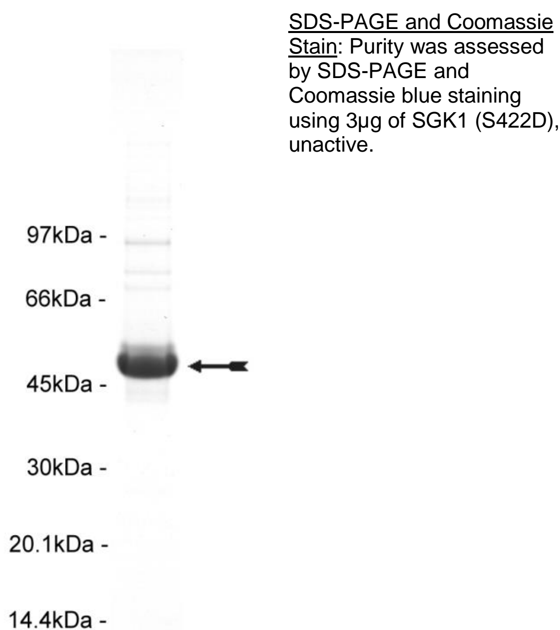
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: Unactive SGK1 (S422D) was activated using PDK1 (cat# 14-452) and the increased activity against crosstide determined. The activation and subsequent assay is described on page two. Results of this assay are shown below.

Active PDK1	Unactive SGK1 (S422D)	ng linear range	Mean cpm	Comments
10ng	500ng	5–20	7864 – 25505	Activated SGK1
2.5µg	7.4µg	50–200	340 – 716	Background

MS Tryptic Fingerprint: Confirmed identity as SGK1 (S422D) 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 SGK1 (S422D), unactive.

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Kinase Cascade Assay Protocol

Stock Solutions:

1. **10 x SGK1 activation buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **5 x SGK1 assay buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
3. **Enzyme Dilution Buffer:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
4. **Magnesium/ATP Cocktail (5 x stock):** 1mM cold ATP and 100mM magnesium acetate.
5. **[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.)
6. **PDK1, active (Catalogue# 14-452):** Use at a final assay concentration of 0.025µM. Prepare a 0.0147mg/ml stock and add 2.5µl of stock per assay point.
7. **SGK1 (S422D), unactive:** Use at a final concentration of 4µM (0.1828mg/ml). Prepare a 0.914mg/ml stock and add 5µl of stock per assay point.
8. **Modified Crosstide (GRPRTSSFAEGKK):** Use a final assay concentration of 30µM. Make a 300µM stock. Add 2.5µl of stock per assay point.

Assay Procedure:

Stage One: *Activation of SGK1 (S422D) by PDK1.*

1. Add 5µl of SGK1 activation buffer to a microcentrifuge tube.
2. Add 5µl of **PDK1, active**.
3. Add **5µl** of **SGK1 (S422D), unactive**.
4. Add 30µl of dH_2O.
5. Add 5µl of magnesium/ATP cocktail.
6. Incubate for 30 minutes at 30°C.
7. Stop reaction by diluting 10–50 fold and placing on ice.

Stage Two: *Phosphorylation of modified crosstide by SGK1 (S422D)* (96 well plate format)

1. Add 5µl of 5 x reaction buffer per assay to wells
2. Add 2.5µl of **modified Crosstide (GRPRTSSFAEGKK)**.
3. Add **2.5µl (5–20ng) SGK1 (S422D), active from Stage One**.
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 twice 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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SGK1 (S422D) Sequence Information

Protein Human SGK1 (S422D)

Tags *N*-terminal 6His

Native sequence I28 of the recombinant protein is equivalent to I60 of human SGK1

Accession number GenBank AF153609

Recombinant SGK1 (S422D) amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGISQ PQEPELMNAN PSPPPSPSQQ INLGPSSNPH
 61 AKPSDFHFLK VIGKGSFGKV LLARHKAEEV FYAVKVLQKK AILKKKEEKH IMSERNVLLK
121 NVKHPFLVGL HFSFQTADKL YFVLDYINGG ELFYHLQRER CFLEPRARFY AAEIASALGY
181 LHSLNIVYRD LKPENILLDS QGHIVLTDFG LCKENIEHNS TTSTFCGTPE YLAPEVLHKQ
241 PYDRTVDWWC LGAVLYEMLY GLPPFYSRNT AEMYDNILNK PLQLKPNITN SARHLLEGLL
301 QKDRTKRLGA KDDFMEIKSH VFFSLINWDD LINKKITPPF NPNVSGPNDL RHFDPEFTEE
361 PVPNSIGKSP DSVLVTASVK EAAEAFLGFD YAPPTDSFL
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Recombinant SGK1 (S422D) nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg gatctcccaa cctcaggagc ctgagcttat gaatgccaac
 121 ccttctcctc caccaagtcc ttctcagcaa atcaaccttg gcccgtcgtc caatcctcat
 181 gctaaaccat ctgactttca cttcttgaaa gtgatcggaa agggcagttt tggaaaggtt
 241 cttctagcaa gacacaaggc agaagaagtg ttctatgcag tcaaagtttt acagaagaaa
 301 gcaatcctga aaaagaaaga ggagaagcat attatgtcgg agcggaatgt tctgttgaag
 361 aatgtgaagc acccttttct ggtgggcctt cacttctctt tccagactgc tgacaaattg
 421 tactttgtcc tagactacat taatggtgga gagttgttct accatctcca gagggaacgc
 481 tgcttcctgg aaccacgggc tcgtttctat gctgctgaaa tagccagtgc cttgggctac
 541 ctgcattcac tgaacatcgt ttatagagac ttaaaaccag agaatatttt gctagattca
 601 cagggacaca ttgtccttac tgatttcgga ctctgcaagg agaacattga acacaacagc
 661 acaacatcca ccttctgtgg cacgccggag tatctcgcac ctgaggtgct tcataagcag
 721 ccttatgaca ggactgtgga ctggtggtgc ctgggagctg tcttgtatga gatgctgtat
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 901 cagaaggaca ggacaaagcg gctcggggcc aaggatgact tcatggagat taagagtcat
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1021 aacccaaatg tgagtgggcc caacgaccta cggcactttg accccgagtt taccgaagag
1081 cctgtcccca actccattgg caagtcccct gacagcgtcc tcgtcacagc cagcgtcaag
1141 gaagctgccg aggctttcct aggctttgac tatgcgcctc ccacggactc tttcctctga
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