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Certificate of Analysis

SGK3, unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-648, 14-648-K, 14-648M
Parent Lot # 31110U

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 unactive, amino acids 119–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 62.7% by SDS-PAGE and Coomassie blue staining. MW = 46.8kDa.

Specific Activity (Parent lot# 31110U): As provided, this lot demonstrated 18% of maximum activity. Activated by phosphorylation with PDK1 (cat# 14-452).

Formulation: 0.49mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 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.

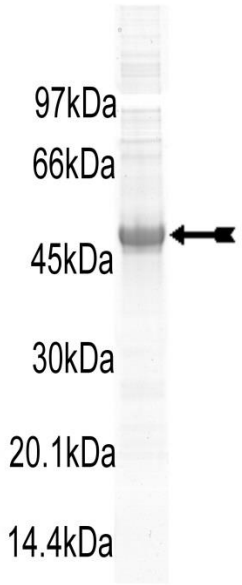
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: Unactive SGK3 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 SGK3	Mean cpm	Comments
36ng	None	1773	Background
None	22.8ng	6657	Background
36ng	22.8ng	7256	Kinase activity

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, unactive.

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Kinase Cascade Assay Protocol

Stock Solutions:

1. **10 x SGK3 Activation Buffer:** 500mM Tris/HCl, pH7.5, 1mM EGTA, 1% 2-mercaptoethanol
2. **5 x SGK3 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):** 500µM cold ATP and 50mM 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 5µl of stock per assay point.
7. **SGK3, unactive:** Use at a final concentration of 2µM (0.0936mg/ml). Prepare a 0.468mg/ml stock and add 5µl of stock per assay point.
8. **Modified Crosstide(GRPRTSSFAEGKK):** Use a final assay concentration of 250µM. Make a 2.5mM stock. Add 2.5µl of stock per assay point.

Assay Procedure:

Stage One: *Activation of SGK3 by PDK1*

1. Add 5µl of 10 x SGK3 activation buffer to a microcentrifuge tube.
2. Add **5µl of SGK3, unactive**.
3. Add 5µl of PDK1, active.
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 SGK3* (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 (1–9ng) SGK3,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 15µl 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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SGK3 Sequence Information

Protein Human SGK3

Tags *N*-Terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M119 of human SGK3

Accession number GenBank NM_013257

Recombinant SGK3 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MDSPKHQSDP SEDEDERSSQ KLHSTSQNIN
  61 LGPSGNPHAK PTDFDFLKVI GKGSFGKVLL AKRKLDGKFY AVKVLQKKIV LNRKEQKHIM
 121 AERNVLLKNV KHPFLVGLHY SFQTTEKLYF VLDFVNGGEL FFHLQRERSF PEHRARFYAA
 181 EIASALGYLH SIKIVYRDLK PENILLDSVG HVVLTDFGLC KEGIAISDTT TTFCGTPEYL
 241 APEVIRKQPY DNTVDWWCLG AVLYEMLYGL PPFYCRDVAE MYDNILHKPL SLRPGVSLTA
 301 WSILEELLEK DRQNRLGAKE DFLEIQNHPF FESLSWADLV QKKIPPPFNP NVAGPDDIRN
 361 FDTAFTEETV PYSVCVSSDY SIVNASVLEA DDAFVGFSYA PPSEDLFL
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Recombinant SGK3 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atggacagtc caaaacacca gtcagatcca
 121 tctgaagatg aggatgaaag aagttctcag aagctacact ctacctcaca gaacatcaac
 181 ctgggaccgt ctggaaatcc tcatgccaaa ccaactgact ttgatttctt aaaagttatt
 241 ggaaaaggca gctttggcaa ggttcttctt gcaaaacgga aactggatgg aaaattttat
 301 gctgtcaaag tgttacagaa aaaaatagtt ctcaacagaa aagagcaaaa acatattatg
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 481 tttttccact tacaaagaga acggtccttt cctgagcaca gagctaggtt ttacgctgct
 541 gaaattgcta gtgcattggg ttacttacat tccatcaaaa tagtatacag agacttgaaa
 601 ccagaaaata ttcttttgga ttcagtagga catgttgtct taacagattt tggggctttgt
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 721 gcacctgaag taattagaaa acagccctat gacaatactg tagattggtg gtgccttggg
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 901 tggtccattc tggaagaact cctagaaaaa gacaggcaaa atcgacttgg tgccaaggaa
 961 gactttcttg aaattcagaa tcatcctttt tttgaatcac tcagctgggc tgaccttgta
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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Reviewed and approved by site quality representative.