eurofins | Discovery Services

Certificate of Analysis

SGK2, unactive

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-636, 14-636-K, 14-636M
Parent Lot # 30942U

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 SGK2, amino acids 54–end, containing the mutation S416D. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 73% by SDS-PAGE and Coomassie blue staining. MW = 45.7kDa.

Specific Activity (Parent lot# 30942U): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with PDK1 (cat# 14-452).

Formulation: 1.39mg/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.

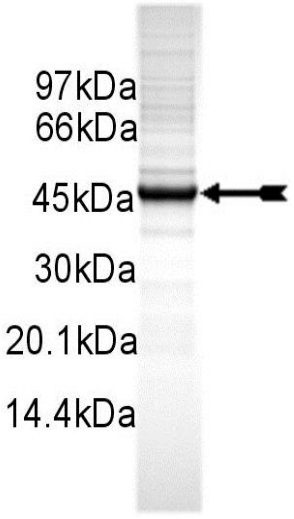
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Activation Assay: Unactive SGK2 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 SGK2	Mean cpm	Comments
36ng	None	160	Background
None	22.8ng	15	Background
36ng	22.8ng	23111	Kinase activity

MS Tryptic Fingerprint: Confirmed identity as SGK2 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 SGK2, unactive.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

Kinase Cascade Assay Protocol

Stock Solutions:

1. **10 x SGK2 activation buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1% 2-mercaptoethanol.
2. **5 x SGK2 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 (Catalogue# 20-113):** 20mM MOPS, pH7.2, 5mM EGTA, 25mM β-glycerophosphate, 1mM Na_3VO_4, 1mM dithiothreitol, 75mM $MgCl_2$, and 0.5mM ATP.
5. **[γ-^{32}P]ATP:** Stock 1mCi/100µl (3000Ci/mmol, obtained from PerkinElmer, Catalogue# BLU002A). Make 10µl aliquots (100µCi/vial). Before starting the assay, dilute an aliquot with 90µl of Magnesium/ATP Cocktail.
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. **SGK2, unactive:** Use at a final concentration of 5µM (0.228mg/ml). Prepare a 1.14mg/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 SGK2 by PDK1.*

1. Add 2.5µl of SGK2 activation buffer to a microcentrifuge tube.
2. Add 2.5µl of **PDK1, active**.
3. Add **5µl** of **SGK2, unactive**.
4. Add 10µl of dH_2O.
5. Add 5µl of magnesium/ATP Cocktail.
6. Incubate for 45 minutes at 30°C.
7. Stop reaction by diluting 10-50 fold and placing on ice.

Stage Two: *Phosphorylation of modified crosstide by SGK2* (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 (11.4–57ng) SGK2, active** from **Stage One** reaction product.
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 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.

eurofins | Discovery Services

Certificate of Analysis

SGK2 Sequence Information

Protein Human SGK2

Tags *N*-terminal 6His

Native sequence L30 of the recombinant protein is equivalent to L54 of human SGK2

Accession number GenBank NM_016276. The recombinant protein contains an amino acid substitution S416D with reference to the native sequence. The S416D mutation in the beta isoform is analogous to the S356D substitution in the alpha isoform and S422D in SGK1, both of which are reported to increase activity.

Recombinant SGK2 amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMGIQL PDHCYRMNSS PAGTPSPQPS RANGNINLGP
 61 SANPNAQPTD FDFLKVIGKG NYGKVLLAKR KSDGAFYAVK VLQKKSILKK KEQSHIMAER
121 SVLLKNVRHP FLVGLRYSFQ TPEKLYFVLD YVNGGELFFH LQRERRFLEP RARFYAAEVA
181 SAIGYLHSLN IIYRDLKPEN ILLDCQGHVV LTDFGLCKEG VEPEDTTSTF CGTPEYLAPE
241 VLRKEPYDRA VDWWCLGAVL YEMLHGLPPF YSQDVSQMYE NILHQPLQIP GGRTVAACDL
301 LQSLLHKDQR QRLGSKADFL EIKNHVFFSP INWDDLYHKR LTPPFNPNVT GPADLKHFDP
361 EFTQEAVSKS IGCTPDTVAS SSGASSAFLG FDYAPEDDDI LDC
```

Recombinant SGK2 nucleotide sequence:

```
   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg gatccagctg cctgatcatt gctacagaat gaactctagc
 121 ccagctggga ccccaagtcc acagccctcc agggccaatg ggaacatcaa cctggggcct
 181 tcagccaacc caaatgccca gcccacggac ttcgacttcc tcaaagtcat cggcaaaggg
 241 aactacggga aggtcctact ggccaagcgc aagtctgatg gggcgttcta tgcagtgaag
 301 gtactacaga aaaagtccat cttaaagaag aaagagcaga gccacatcat ggcagagcgc
 361 agtgtgcttc tgaagaacgt gcggcacccc ttcctcgtgg gcctgcgcta ctccttccag
 421 acacctgaga agctctactt cgtgctcgac tatgtcaacg ggggagagct cttcttccac
 481 ctgcagcggg agcgccggtt cctggagccc cgggccaggt tctacgctgc tgaggtggcc
 541 agcgccattg gctacctgca ctccctcaac atcatttaca gggatctgaa accagagaac
 601 attctcttgg actgccaggg acacgtggtg ctgacggatt ttggcctctg caaggaaggt
 661 gtagagcctg aagacaccac atccacattc tgtggtaccc ctgagtactt ggcacctgaa
 721 gtgcttcgga aagagcctta tgatcgagca gtggactggt ggtgcttggg ggcagtcctc
 781 tacgagatgc tccatggcct gccgcccttc tacagccaag atgtatccca gatgtatgag
 841 aacattctgc accagccgct acagatcccc ggaggccgga cagtggccgc ctgtgacctc
 901 ctgcaaagcc ttctccacaa ggaccagagg cagcggctgg gctccaaagc agactttctt
 961 gagattaaga accatgtatt cttcagcccc ataaactggg atgacctgta ccacaagagg
1021 ctaactccac ccttcaaccc aaatgtgaca ggacctgctg acttgaagca ttttgaccca
1081 gagttcaccc aggaagctgt gtccaagtcc attggctgta cccctgacac tgtggccagc
1141 agctctgggg cctcaagtgc attcctggga tttgactatg cgccagagga tgatgacatc
1201 ttggattgct ag
```

Reviewed and approved by site quality representative.