Standard Operating Procedure

Preparation of active SGK1

Enzyme description:- **Active SGK1 Δ1-59 S422D**

Source:- Recombinant

Expression system:- Baculovirus expression vector system (BEVS)/Insect cells

Tag:- His(6)

Purification method:- Ni^{2+}-NTA agarose.

Expression level:- 3-5 mg/L

Molecular mass:- 48 kDa by SDS-PAGE

Purity:- >90%

Contaminants:- The preparation also contains several minor contaminating proteins.

Activation protocol:-

SGK1 (0.2 mg/ml - 4 μM) is activated in 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 0.1 % 0.1 β-mercaptoethanol, 10mM magnesium acetate, 0.1mM ATP with 3.3 μg/ml PDK1 in at 30°C for 30 min. Following activation the preparation is made 400 mM NaCl by addition of 1/5 volume of 50 mM Tris/HCl pH 7.5, 2 M NaCl, 0.1 mM EGTA, 0.1 % β-mercaptoethanol and the PDK1 is removed by chromatography of the preparation on Heparin Sepharose (PDK1 binds very strongly to Heparin – SGK1 is in the flowthrough). The active SGK1 is then re-purified on Ni-NTA agarose to concentrate the enzyme. The active SGK1 is then eluted from the column, dialysed into enzyme storage buffer and snap frozen in liquid nitrogen prior to storage at –70°C.

Enzyme storage buffer:-

50 mM Tris/HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β–mercaptoethanol, 0.2 mM PMSF,1 mM Benzamidine.

Storage temperature:- Aliquot, snap freeze and store at -70°C.

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CLONE DATA SHEET –human SGK1

Protein	Human SGK1 Δ1-59 S422D
Accession number	NM_005627
Tags	His
Bacterially-expressed protein	MSYYHHHHHHDYDIPTTENLVFQGAMG**ISQPQEPELMNANP SPPPSPSQQINLGPSSNPHAKPSDFHFLKVIGKGSFGKVLL ARHKAEEVFYAVKVLQKKAILKKKEEKHIMSERNVLLKNVK HPFLVGLHFSFQTADKLYFVLDYINGGELFYHLQRERCFLE PRARFYAAEIASALGYLHSLNIVYRDLKPENILLDSQGHIV LTDFGLCKENIEHNSTTSTFCGTPEYLAPEVLHKQPYDRTV DWWCLGAVLYEMLYGLPPFYSRNTAEMYDNILNKPLQLKPN ITNSARHLLEGLLQKDRTKRLGAKDDFMEIKSHVFFSLINW DDLINKKITPPFNPNVSGPNDLRHFDPEFTEEPVPNSIGKS PDSVLVTASVKEAAEAFLGFDYAPPTDSFL**
Native sequence	I28 of the fusion protein is equivalent to I60 of SGK1. Serine 422 mutated to Aspartate. This mutation results in a mimic of phosphorylation at the 'PDK2 site' and therefore an artificially elevated activity following PDK1 activation.
Protease cleavage site	ENLYFQ (rTEV protease) residues18 – 23 of His_6 tagged protein.
Cloning sites	BamHI/Not1 sites of pFastBAC-HTc with BglII/NotI fragment of SGK1.

Nucleotide sequence of ORF in baculovirus

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACG
ACCGAAAACCTGTATTTTCAGGGCGCCATGGGGATCTCCCAACCTCAG
GAGCCTGAGCTTATGAATGCCAACCCTTCTCCTCCACCAAGTCCTTCT
CAGCAAATCAACCTTGGCCCGTCGTCCAATCCTCATGCTAAACCATCT
GACTTTCACTTCTTGAAAGTGATCGGAAAGGGCAGTTTTGGAAAGGTT
CTTCTAGCAAGACACAAGGCAGAAGAAGTGTTCTATGCAGTCAAAGTT
TTACAGAAGAAAGCAATCCTGAAAAAGAAAGAGGAGAAGCATATTATG
TCGGAGCGGAATGTTCTGTTGAAGAATGTGAAGCACCCTTTCCTGGTG
GGCCTTCACTTCTCTTTCCAGACTGCTGACAAATTGTACTTTGTCCTA
GACTACATTAATGGTGGAGAGTTGTTCTACCATCTCCAGAGGGAACGC
TGCTTCCTGGAACCACGGGCTCGTTTCTATGCTGCTGAAATAGCCAGT
GCCTTGGGCTACCTGCATTCACTGAACATCGTTTATAGAGACTTAAAA
CCAGAGAATATTTTGCTAGATTCACAGGGACACATTGTCCTTACTGAT
TTCGGACTCTGCAAGGAGAACATTGAACACAACAGCACAACATCCACC
TTCTGTGGCACGCCGGAGTATCTCGCACCTGAGGTGCTTCATAAGCAG
CCTTATGACAGGACTGTGGACTGGTGGTGCCTGGGAGCTGTCTTGTAT
GAGATGCTGTATGGCCTGCCGCCTTTTTATAGCCGAAACACAGCTGAA
ATGTACGACAACATTCTGAACAAGCCTCTCCAGCTGAAACCAAATATT
ACAAATTCCGCAAGACACCTCCTGGAGGGCCTCCTGCAGAAGGACAGG
ACAAAGCGGCTCGGGGCCAAGGATGACTTCATGGAGATTAAGAGTCAT
GTCTTCTTCTCCTTAATTAACTGGGATGATCTCATTAATAAGAAGATT
ACTCCCCCTTTTAACCCAAATGTGAGTGGGCCCAACGAGCTACGGCAC
TTTGACCCCGAGTTTACCGAAGAGCCTGTCCCCAACTCCATTGGCAAG
TCCCCTGACAGCGTCCTCGTCACAGCCAGCGTCAAGGAAGCTGCCGAG
GCTTTCCTAGGCTTTGACTATGCGCCTCCCACGGACTCTTTCCTCTGA