Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active SGK1 S422D [60 – 431]

Enzyme description:- SGK1 S422D [60 - 431]

Clone number:- DU 1737

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 3-5 mg/L

Calculated molecular mass:- 45, 475 daltons

Purity:- >90 %

Activation protocol:-

SGK1 (4 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM ATP with 3.3 µg/ml GST-PDK1 [DU 954] at 30 °C for 30 min. Following activation, SGK1 is re-purified by Ni^{2+}-NTA agarose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1% 2−mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
CROSStide peptide (GRPRTSSFAEG) Final concentration: 30 µM

Specific activity range:- 500 – 1000 U/mg

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Clone Data Sheet

SGK1 S422D [60 - 431]

Protein	SGK1 S422D [60 – 431]
Clone number	DU 1737
Species	Human
Accession number	NM_005627
Tags	N-terminal His(6)
Bacterially expressed protein	MSYYHHHHHHDYDIPTTENLVFQGAMG**ISQPQEPELMNANPSPPPSPS QQINLGPSSNPHAKPSDFHFLKVIGKGSFGKVLLARHKAEEVFYAVKV LQKKAILKKKEEKHIMSERNVLLKNVKHPFLVGLHFSFQTADKLYFVL DYINGGELFYHLQRERCFLEPRARFYAAEIASALGYLHSLNIVYRDLK PENILLDSQGHIVLTDFGLCKENIEHNSTTSTFCGTPEYLAPEVLHKQ PYDRTVDWWCLGAVLYEMLYGLPPFYSRNTAEMYDNILNKPLQLKPNI TNSARHLLEGLLQKDRTKRLGAKDDFMEIKSHVFFSLINWDDLINKKI TPPFNPNVSGPNDLRHFDPEFTEEPVPNSIGKSPDSVLVTASVKEAAE AFLGFDYAPPTDSFL**
Native sequence	Amino acids I60 –L431 (end) of human SGK1. Residue I28 of the fusion protein is equivalent to I60 of the native enzyme. The enzyme has a S422D mutation to mimic phosphorylation of the PDK2 site. Residue S422 is equilivalent to D390 of the fusion protein. The His(6) tag is located at residues 5 - 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24
Cloning sites	*Bam*HI and *Not*1 sites of pFastBAC-HTc

Nucleotide sequence of insert

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