Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Sphingosine Kinase 1 [1 - 384]

Enzyme description:- SPHK1 [1 - 384]

Clone number:- DU 12303

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic 47, 299.12 daltons
Average Mass 47, 329.83 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.52

Purity:- >80 %

Activation protocol:- Constitutively active

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:- ADP Glo

Assay buffer:-
12.5mM Glycine-NaOH (pH 8.5), 50mM KCl, 2.5mM MgCl2
Substrate:-
Sphingosine Final concentration: 10 μM

Specific activity range:- To be determined

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Clone Data Sheet

Sphingosine Kinase 1 [1 – 384]

Protein	SPHK1 [1 - 384]
Clone number	DU 12303
Species	Human
Accession number	AF200328.1
Tags	N-terminal His(6)
Baculovirus Expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGSGIQRPTSTSSLVAAA**MDPAGG PRGVLPRPCRVLVLLNPRGGKGKALQLFRSHVQPLLAEAEISFTLMLTE RRNHARELVRSEELGRWDALVVMSGDGLMHEVVNGLMERPDWETAIQKP LCSLPAGSGNALAASLNHYAGYEQVTNEDLLTNCTLLLCRRLLSPMNLL SLHTASGLRLFSVLSLAWGFIADVDLESEKYRRLGEMRFTLGTFLRLAA LRTYRGRLAYLPVGRVGSKTPASPVVVQQGPVDAHLVPLEEPVPSHWTV VPDEDFVLVLALLHSHLGSEMFAAPMGRCAAGVMHLFYVRAGVSRAMLL RLFLAMEKGRHMEYECPYLVYVPVVAFRLEPKDGKGVFAVDGELMVSEA VQGQVHPNYFWMVSGCVEPPPSWKPQQMPPPEEPL**
Native sequence	Amino acids M1 – L384 (end) of human SPHK1. Residue M44 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24
Cloning sites	*Not*1 of pFastBac HTb

Nucleotide of sequence of insert

gcggccgcgATGGATCCAGCGGGCGGCCCCCGGGGCGTGCTCCCGCGGC
CCTGCCGCGTGCTGGTGCTGCTGAACCCGCGCGGCGGCAAGGGCAAGGC
CTTGCAGCTCTTCCGGAGTCACGTGCAGCCCCTTTTGGCTGAGGCTGAA
ATCTCCTTCACGCTGATGCTCACTGAGCGGCGGAACCACGCGCGGGAGC
TGGTGCGGTCGGAGGAGCTGGGCCGCTGGGACGCTCTGGTGGTCATGTC
TGGAGACGGGCTGATGCACGAGGTGGTGAACGGGCTCATGGAGCGGCCT
GACTGGGAGACCGCCATCCAGAAGCCCCTGTGTAGCCTCCCAGCAGGCT
CTGGCAACGCGCTGGCAGCTTCCTTGAACCATTATGCTGGCTATGAGCA
GGTCACCAATGAAGACCTCCTGACCAACTGCACGCTATTGCTGTGCCGC
CGGCTGCTGTCACCCATGAACCTGCTGTCTCTGCACACGGCTTCGGGGC
TGCGCCTCTTCTCTGTGCTCAGCCTGGCCTGGGGCTTCATTGCTGATGT
GGACCTAGAGAGTGAGAAGTATCGGCGTCTGGGGGAGATGCGCTTCACT
CTGGGCACCTTCCTGCGTCTGGCAGCCCTGCGCACCTACCGCGGCCGAC
TGGCCTACCTCCCTGTAGGAAGAGTGGGTTCCAAGACACCTGCCTCCCC
CGTTGTGGTCCAGCAGGGCCCGGTAGATGCACACCTTGTGCCACTGGAG
GAGCCAGTGCCCTCTCACTGGACAGTGGTGCCCGACGAGGACTTTGTGC
TAGTCCTGGCACTGCTGCACTCGCACCTGGGCAGTGAGATGTTTGCTGC
ACCCATGGGCCGCTGTGCAGCTGGCGTCATGCATCTGTTCTACGTGCGG
GCGGGAGTGTCTCGTGCCATGCTGCTGCGCCTCTTCCTGGCCATGGAGA
AGGGCAGGCATATGGAGTATGAATGCCCCTACTTGGTATATGTGCCCGT
GGTCGCCTTCCGTTTGGAGCCCAAGGATGGGAAAGGTGTGTTTGCAGTG
GATGGGGAATTGATGGTTAGCGAGGCCGTGCAGGGCCAGGTGCACCCAA
ACTACTTCTGGATGGTCAGCGGTTGCGTGGAGCCCCCGCCCAGCTGGAA
GCCCCAGCAGATGCCACCGCCAGAAGAGCCCTTAtgagcggccgc