

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Sphingosine Kinase 2 [1 - 654]

<u>Enzyme description:-</u>	SPHK2 [1 - 654]
<u>Clone number:-</u>	DU 12551
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	5 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	72, 543.11 daltons
Average Mass	72, 588.85 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.36
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
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	
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	ADP Glo
<u>Assay buffer:-</u>	
12.5mM Glycine-NaOH (pH 8.5), 50mM KCl, 2.5mM MgCl ₂	
<u>Substrate:-</u>	
Sphingosine	Final concentration: 10 µM
<u>Specific activity range:-</u>	To be determined

Division of Signal Transduction Therapy

Clone Data Sheet

Sphingosine Kinase 2 [1 – 654]

Protein SPHK2 [1 - 654]

Clone number DU 12551

Species Human

Accession number NM_020126.3

Tags N-terminal His(6)

Baculovirus
Expressed protein MSYYHHHHHHHDYDIPTTENLYFQGAMGSMNGHLEAEEQQDQRPDQELTG
SWG HGP RSTLVRAKAMAPPPPLAASTPLLHGEFGSYPARGPRFALTLT
SQALHIQRLRPKPEARPRGGLVPLAEVSGCCTLRSPSDSAAAYFCIYT
YPRGRRGARRRATRTFRADGAATYEENRAEAQRWATALTCLLRGLPLPG
DGEITPDLLPRPPRLLLLVPFVGGRGLAWQWCKNHVLP MISEAGLSFNL
IQTERQNHARELVQGLSLSEWDGIVTVSGDGLLHEVLNGLLDRPDWEEA
VKMPVGILPCGSGNALAGAVNQHG GFEPALGLDLLNCSLLLCRGGGHP
LDLLSVTLASGSRCSFLSVAWG FVSDVDIQSERFRALGSARFTLGTVL
GLATLHTYRGRLSYLPATVEPASPTPAHSLPRAKSELTLPDPAPMAH
SPLHRVSVDLPLPLPQPALASPGSPEPLPILSLNGGGPELAGDWGGAGD
APLSPDPLLSSPPGSPKAALHSPVSEGAPVI PPSSGLPLPTPDARVGAS
TCGPPDHLLPPLGTPLPPDWVTLEGDFVLM LAISPSHLGADLVAAPHAR
FDDGLVHLCWVRSGISRAALLRLFLAMERGS HFSLGCPQLGYAAARA FR
LEPLTPRGVLTVDGEQVEYGPLQAQMHPGIGTLLTGPPGCPGREG

Native sequence Amino acids M1 – P654 (end) of human SPHK2.
Residue M29 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 BamH1 and NotI of pFastBac HTb

Division of Signal Transduction Therapy

Nucleotide of sequence of insert

ggatccATGAATGGACACCTTGAAGCAGAGGAGCAGCAGGACCAGAGGC
CAGACCAGGAGCTGACCGGGAGCTGGGGCCACGGGCCTAGGAGCACCCCT
GGTCAGGGCTAAGGCCATGGCCCCGCCCCACCGCCACTGGCTGCCAGC
ACCCCGCTCCTCCATGGCGAGTTTGGCTCCTACCCAGCCCAGAGGCCAC
GCTTTGCCCTCACCCTTACATCGCAGGCCCTGCACATACAGCGGCTGCG
CCCCAACCTGAAGCCAGGCCCGGGGTGGCCTGGTCCCCTTGCCGAG
GTCTCAGGCTGCTGCACCCTGCGAAGCCGAGCCCCCTCAGACTCAGCGG
CCTACTTCTGCATCTACACCTACCCTCGGGGCCGGCGCGGGGCCGGCG
CAGAGCCACTCGCACCTTCCGGGCAGATGGGGCCGCCACCTACGAAGAG
AACCCTGCCGAGGCCAGCGCTGGGCCACTGCCCTCACCTGTCTGCTCC
GAGGACTGCCACTGCCCGGGATGGGGAGATCACCCCTGACCTGCTACC
TCGGCCGCCCCGGTTGCTTCTATTGGTCAATCCCTTTGGGGGTGCGGGC
CTGGCCTGGCAGTGGTGTAAAGAACCGTGCTTCCCATGATCTCTGAAG
CTGGGCTGTCCTTCAACCTCATCCAGACAGAACGACAGAACCACGCCCG
GGAGCTGGTCCAGGGGCTGAGCCTGAGTGAGTGGGATGGCATCGTCAAG
GTCTCGGGAGACGGGCTGCTCCATGAGGTGCTGAACGGGCTCCTAGATC
GCCCTGACTGGGAGGAAGCTGTGAAGATGCCTGTGGGCATCCTCCCCTG
CGGCTCGGGCAACGCGCTGGCCGGAGCAGTGAACCAGCACGGGGGATTT
GAGCCAGCCCTGGGCCTCGACCTGTTGCTCAACTGCTCACTGTTGCTGT
GCCGGGGTGGTGGCCACCCACTGGACCTGCTCTCCGTGACGCTGGCCTC
GGGCTCCCGCTGTTTCTCCTTCCCTGTCTGTGGCCTGGGGCTTCGTGTCA
GATGTGGATATCCAGAGCGAGCGCTTCAGGGCCTTGGGCAGTGCCCGCT
TCACACTGGGCACGGTGCTGGGCCTCGCCACACTGCACACCTACCGCGG
ACGCCTCTCCTACCTCCCCGCCACTGTGGAACCTGCCTCGCCACCCCT
GCCCATAGCCTGCCTCGTGCCAAGTCGGAGCTGACCCTAACCCCAGACC
CAGCCCCGCCCATGGCCCACTCACCCCTGCATCGTTCTGTGTCTGACCT
GCCTCTTCCCCTGCCCCAGCCTGCCCTGGCCTCTCCTGGCTCGCCAGAA
CCCCTGCCCATCCTGTCCCTCAACGGTGGGGGCCAGAGCTGGCTGGGG
ACTGGGGTGGGGCTGGGGATGCTCCGCTGTCCCCGGACCCACTGCTGTC
TTCACCTCCTGGCTCTCCCAAGGCAGCTCTACACTCACCCGTCTCCGAA
GGGGCCCCCGTAATTCCCCCATCCTCTGGGCTCCCACTTCCCACCCCTG
ATGCCCGGGTAGGGGCCTCCACCTGCGGCCCGCCGACCACCTGCTGCC
TCCGCTGGGCACCCCGCTGCCCCAGACTGGGTGACGCTGGAGGGGGAC
TTTGTGCTCATGTTGGCCATCTCGCCCAGCCACCTAGGCGCTGACCTGG
TGGCAGCTCCGCATGCGCGCTTCGACGACGGCCTGGTGCACCTGTGCTG
GGTGCGTAGCGGCATCTCGCGGGCTGCGCTGCTGCGCCTTTTCTTGCC
ATGGAGCGTGGTAGCCACTTCAGCCTGGGCTGTCCGCAGCTGGGCTACG
CCGCGGCCCGTGCTTCCGCCTAGAGCCGCTCACACCACGCGGCGTGCT
CACAGTGGACGGGGAGCAGGTGGAGTATGGGCCGCTACAGGCACAGATG
CACCTGGCATCGGTACACTGCTCACTGGGCCTCCTGGCTGCCCCGGGC
GGGAGCCCTgagcggccgc