Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Sphingosine Kinase 2 [1 - 654]

Enzyme description:- SPHK2 [1 - 654]

Clone number:- DU 12551

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 72, 543.11 daltons
Average Mass 72, 588.85 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.36

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:- Kinase Glo

Assay buffer:-
50 mM Tris-HCl pH 7.5, 200 mM KCl, 1 mM DTT, 1 mM EGTA, 5 mM $MgCl_2$

Substrate:-
Sphingosine Final concentration: 10 μM

Specific activity range:- To be determined

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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

MSYYHHHHHHDYDIPTTENLYFQGAMGS**MNGHLEAEEQQDQRPDQELTG
SWGHGPRSTLVRAKAMAPPPPPLAASTPLLHGEFGSYPARGPRFALTLT
SQALHIQRLRPKPEARPRGGLVPLAEVSGCCTLRSRSPSDSAAYFCIYT
YPRGRRGARRRATRTFRADGAATYEENRAEAQRWATALTCLLRGLPLPG
DGEITPDLLPRPPRLLLLVNPFGGRGLAWQWCKNHVLPMISEAGLSFNL
IQTERQNHARELVQGLSLSEWDGIVTVSGDGLLHEVLNGLLDRPDWEEA
VKMPVGILPCGSGNALAGAVNQHGGFEPALGLDLLLNCSLLLCRGGGHP
LDLLSVTLASGSRCFSFLSVAWGFVSDVDIQSERFRALGSARFTLGTVL
GLATLHTYRGRLSYLPATVEPASPTPAHSLPRAKSELTLTPDPAPPMAH
SPLHRSVSDLPLPLPQPALASPGSPEPLPILSLNGGGPELAGDWGGAGD
APLSPDPLLSSPPGSPKAALHSPVSEGAPVIPPSSGLPLPTPDARVGAS
TCGPPDHLLPPLGTPLPPDWVTLEGDFVLMLAISPSHLGADLVAAPHAR
FDDGLVHLCWVRSGISRAALLRLFLAMERGSHFSLGCPQLGYAAARAFR
LEPLTPRGVLTVDGEQVEYGPLQAQMHPGIGTLLTGPPGCPGREP**

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 *Bam*H1 and *Not*1 of pFastBac HTb

Nucleotide of sequence of insert

ggatccATGAATGGACACCTTGAAGCAGAGGAGCAGCAGGACCAGAGGC
CAGACCAGGAGCTGACCGGGAGCTGGGGCCACGGGCCTAGGAGCACCCT
GGTCAGGGCTAAGGCCATGGCCCCGCCCCCACCGCCACTGGCTGCCAGC
ACCCCGCTCCTCCATGGCGAGTTTGGCTCCTACCCAGCCCGAGGCCCAC
GCTTTGCCCTCACCCTTACATCGCAGGCCCTGCACATACAGCGGCTGCG
CCCCAAACCTGAAGCCAGGCCCCGGGGTGGCCTGGTCCCGTTGGCCGAG
GTCTCAGGCTGCTGCACCCTGCGAAGCCGCAGCCCCTCAGACTCAGCGG
CCTACTTCTGCATCTACACCTACCCTCGGGGCCGGCGCGGGGCCCGGCG
CAGAGCCACTCGCACCTTCCGGGCAGATGGGGCCGCCACCTACGAAGAG
AACCGTGCCGAGGCCCAGCGCTGGGCCACTGCCCTCACCTGTCTGCTCC
GAGGACTGCCACTGCCCGGGGATGGGGAGATCACCCCTGACCTGCTACC
TCGGCCGCCCCGGTTGCTTCTATTGGTCAATCCCTTTGGGGGTCGGGGC
CTGGCCTGGCAGTGGTGTAAGAACCACGTGCTTCCCATGATCTCTGAAG
CTGGGCTGTCCTTCAACCTCATCCAGACAGAACGACAGAACCACGCCCG
GGAGCTGGTCCAGGGGCTGAGCCTGAGTGAGTGGGATGGCATCGTCACG
GTCTCGGGAGACGGGCTGCTCCATGAGGTGCTGAACGGGCTCCTAGATC
GCCCTGACTGGGAGGAAGCTGTGAAGATGCCTGTGGGCATCCTCCCCTG
CGGCTCGGGCAACGCGCTGGCCGGAGCAGTGAACCAGCACGGGGGATTT
GAGCCAGCCCTGGGCCTCGACCTGTTGCTCAACTGCTCACTGTTGCTGT
GCCGGGGTGGTGGCCACCCACTGGACCTGCTCTCCGTGACGCTGGCCTC
GGGCTCCCGCTGTTTCTCCTTCCTGTCTGTGGCCTGGGGCTTCGTGTCA
GATGTGGATATCCAGAGCGAGCGCTTCAGGGCCTTGGGCAGTGCCCGCT
TCACACTGGGCACGGTGCTGGGCCTCGCCACACTGCACACCTACCGCGG
ACGCCTCTCCTACCTCCCCGCCACTGTGGAACCTGCCTCGCCCACCCCT
GCCCATAGCCTGCCTCGTGCCAAGTCGGAGCTGACCCTAACCCCAGACC
CAGCCCCGCCCATGGCCCACTCACCCCTGCATCGTTCTGTGTCTGACCT
GCCTCTTCCCCTGCCCCAGCCTGCCCTGGCCTCTCCTGGCTCGCCAGAA
CCCCTGCCCATCCTGTCCCTCAACGGTGGGGGCCCAGAGCTGGCTGGGG
ACTGGGGTGGGGCTGGGGATGCTCCGCTGTCCCCGGACCCACTGCTGTC
TTCACCTCCTGGCTCTCCCAAGGCAGCTCTACACTCACCCGTCTCCGAA
GGGGCCCCCGTAATTCCCCCATCCTCTGGGCTCCCACTTCCCACCCCTG
ATGCCCGGGTAGGGGCCTCCACCTGCGGCCCGCCCGACCACCTGCTGCC
TCCGCTGGGCACCCCGCTGCCCCCAGACTGGGTGACGCTGGAGGGGGAC
TTTGTGCTCATGTTGGCCATCTCGCCCAGCCACCTAGGCGCTGACCTGG
TGGCAGCTCCGCATGCGCGCTTCGACGACGGCCTGGTGCACCTGTGCTG
GGTGCGTAGCGGCATCTCGCGGGCTGCGCTGCTGCGCCTTTTCTTGGCC
ATGGAGCGTGGTAGCCACTTCAGCCTGGGCTGTCCGCAGCTGGGCTACG
CCGCGGCCCGTGCCTTCCGCCTAGAGCCGCTCACACCACGCGGCGTGCT
CACAGTGGACGGGGAGCAGGTGGAGTATGGGCCGCTACAGGCACAGATG
CACCCTGGCATCGGTACACTGCTCACTGGGCCTCCTGGCTGCCCGGGGC
GGGAGCCCtgagcggccgc