Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active STK33 [1 - 514]

Enzyme description:- STK33 [1 – 514]

Clone number:- DU 30160

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 84, 675.05 daltons
Average Mass 84, 729.07 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.23

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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

Substrate:-
AALVRQMSVAFFFKKKKK Final concentration: 300 μM

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Clone Data Sheet

STK33 [1 - 514]

Protein	STK33 [1 - 514]
Clone number	DU 30160
Species	Human
Accession number	BC031231.1
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MADSGLDKK** **STKCPDCSSASQKDVLCVCSSKTRVPPVLVVEMSQTSSIGSAESLISL** **ERKKEKNINRDITSRKDLPSRTSNVERKASQQQWGRGNFTEGKVPHIR** **IENGAAIEEIYTFGRILGKGSFGIVIEATDKETETKWAIKKVNKEKAG** **SSAVKLLEREVNILKSVKHEHIIHLEQVFETPKKMYLVMELCEDGELK** **EILDRKGHFSENETRWIIQSLASAIAYLHNNDIVHRDLKLENIMVKSS** **LIDDNNEINLNIKVTDFGLAVKKQSRSEAMLQATCGTPIYMAPEVISA** **HDYSQQCDIWSIGVVMYMLLRGEPPFLASSEEKLFELIRKGELHFENA** **VWNSISDCAKSVLKQLMKVDPAHRITAKELLDNQWLTGNKLSSVRPTN** **VLEMMKEWKNNPESVEENTTEEKNKPSTEEKLKSYQPWGNVPETNYTS** **DEEEEKQSTTYEKQFPATSKDNFDMCSSSFTSSKLLPAEIKGEMEKTP** **VTPSQGTATKYPAKSGALSRTKKKL**
Native sequence	Amino acids M1 – L514 of human STK33. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pFB GST 6P

Nucleotide sequence of insert

ggatccATGGCTGATAGTGGCTTAGATAAAAAATCCACAAAATGCCCC
GACTGTTCATCTGCTTCTCAGAAAGATGTACTTTGTGTATGTTCCAGC
AAAACAAGGGTTCCTCCAGTTTTGGTGGTGGAAATGTCACAGACATCA
AGCATTGGTAGTGCAGAATCTTTAATTTCACTGGAGAGAAAAAAAGAA
AAAAATATCAACAGAGATATAACCTCCAGGAAAGATTTGCCCTCAAGA
ACCTCAAATGTAGAGAGAAAAGCATCTCAGCAACAATGGGGTCGGGGC
AACTTTACAGAAGGAAAAGTTCCTCACATAAGGATTGAGAATGGAGCT
GCTATTGAGGAAATCTATACCTTTGGAAGAATATTGGGAAAAGGGAGC
TTTGGAATAGTCATTGAAGCTACAGACAAGGAAACAGAAACGAAGTGG
GCAATTAAAAAAGTGAACAAAGAAAAGGCTGGAAGCTCCGCTGTGAAG
TTACTTGAACGAGAGGTGAACATTCTGAAAAGTGTAAAACATGAACAC
ATCATACATCTGGAACAAGTATTTGAAACGCCAAAGAAAATGTACCTT
GTGATGGAGCTTTGTGAGGATGGAGAACTCAAAGAAATTCTGGATAGG
AAAGGGCATTTCTCAGAGAATGAGACAAGGTGGATCATTCAAAGTCTC
GCATCAGCTATAGCATATCTTCACAATAATGATATTGTACATAGGGAT
CTGAAACTGGAAAATATAATGGTTAAAAGCAGTCTTATTGATGATAAC
AATGAAATAAACTTAAACATAAAGGTGACTGATTTTGGCTTAGCGGTG
AAGAAGCAAAGTAGGAGTGAAGCCATGCTGCAGGCCACATGTGGGACT
CCTATCTATATGGCCCCTGAAGTTATCAGTGCCCACGACTATAGCCAG
CAGTGTGACATTTGGAGCATAGGCGTCGTAATGTACATGTTATTACGT
GGAGAACCACCCTTTTTGGCAAGCTCAGAAGAGAAGCTTTTTGAGTTA
ATAAGAAAAGGAGAACTACATTTTGAAAATGCAGTCTGGAATTCCATA
AGTGACTGTGCTAAAAGTGTTTTGAAACAACTTATGAAAGTAGATCCT
GCTCACAGAATCACAGCTAAGGAACTACTAGATAACCAGTGGTTAACA
GGCAATAAACTTTCTTCGGTGAGACCAACCAATGTATTAGAGATGATG
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TGGGGAAATGTCCCTGAGACCAATTACACTTCAGATGAAGAGGAGGAA
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AACTTTGATATGTGCAGTTCAAGTTTCACATCTAGCAAACTCCTTCCA
GCTGAAATCAAGGGAGAAATGGAGAAAACCCCTGTGACTCCAAGCCAA
GGAACAGCAACCAAGTACCCTGCTAAATCCGGCGCCCTGTCCAGAACC
AAAAAGAAACTCtaagcggccgc