

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active STK33 [1 - 514]

<u>Enzyme description:-</u>	STK33 [1 – 514]
<u>Clone number:-</u>	DU 30160
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	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:-

AALVRQMSVAFFFFKKKKK

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**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEELHYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLE
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSMADSGLDKK
STKCPDCSSASQKDVLCVCSKTRVPPVVLVEMSQTSSIGSAESLISL
ERKKEKNINRDITSRKDLPSRTSNVERKASQQWGRGNFTEGKVPHIR
IENGAAIEEIYTFGRILGKGSFGIVIEATDKETETKWAIKKVNKEKAG
SSAVKLLEREVNILKSVKHEHIHLEQVFETPKMYLVMELCEDGELK
EILDRKGFSENETRWIIQSLASAIAYLHNNDIVHRDLKLENIMVKSS
LIDDNNEINLNIKVTDFGLAVKKQSRSEAMLQATCGTPIYMAPEVISA
HDYSQQCDIWSIGVVMYMLLRGEPFLASSEKLFELIRKSELHFENA
VWNSISDCAKSVLKQLMKVDPAHRITAKELLDNQWLTGNKLSSVRPTN
VLEMMKEWKNNPESVEENTTEEKNKPSTEEKLKSYPWGNVPETNYTS
DEEEKQSTTYEKQFPATSKDNFDMCSSSFTSSKLLPAEIKGEMEKTP
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

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**Nucleotide
sequence of
insert**

ggatccATGGCTGATAGTGGCTTAGATAAAAAATCCACAAAATGCCCC
GACTGTTTCATCTGCTTCTCAGAAAGATGTACTTTGTGTATGTTCCAGC
AAAACAAGGGTTCCTCCAGTTTTGGTGGTGGAAATGTCACAGACATCA
AGCATTGGTAGTGCAGAATCTTTAATTTCACTGGAGAGAAAAAAGAA
AAAAATATCAACAGAGATATAACCTCCAGGAAAGATTTGCCCTCAAGA
ACCTCAAATGTAGAGAGAAAAGCATCTCAGCAACAATGGGGTCTGGGGC
AACTTTACAGAAGGAAAAGTTCTCACATAAGGATTGAGAATGGAGCT
GCTATTGAGGAAATCTATACCTTTGGAAGAATATTGGGAAAAGGGAGC
TTTGGAAATAGTCATTGAAGCTACAGACAAGGAAACAGAAACGAAGTGG
GCAATTA AAAAAGTGAACAAAGAAAAGGCTGGAAGCTCCGCTGTGAAG
TTACTTGAACGAGAGGTGAACATTTCTGAAAAGTGTA AACATGAACAC
ATCATA CATCTGGAACAAGTATTTGAAACGCCAAAGAAAATGTACCTT
GTGATGGAGCTTTGTGAGGATGGAGA ACTCAAAGAAATTTCTGGATAGG
AAAGGGCATTTCTCAGAGAATGAGACAAGGTGGATCATTCAAAGTCTC
GCATCAGCTATAGCATATCTTCACAATAATGATATTGTACATAGGGAT
CTGAAACTGGAAAATATAATGGTTAAAAGCAGTCTTATTGATGATAAC
AATGAAATAAACTTAAACATAAAGGTGACTGATTTTGGCTTAGCGGTG
AAGAAGCAAAGTAGGAGTGAAGCCATGCTGCAGGCCACATGTGGGACT
CCTATCTATATGGCCCCTGAAGTTATCAGTGCCACGACTATAGCCAG
CAGTGTGACATTTGGAGCATAGGCGTCGTAATGTACATGTTATTACGT
GGAGAACCACCCTTTTTGGCAAGCTCAGAAGAGAAGCTTTTTGAGTTA
ATAAGAAAAGGAGA ACTACATTTTGAAAATGCAGTCTGGAATTCATA
AGTGACTGTGCTAAAAGTGTTTTGAAACA ACTTATGAAAGTAGATCCT
GCTCACAGAATCACAGCTAAGGAACTACTAGATAACCAGTGGTTAACA
GGCAATAAACTTTCTTCGGTGAGACCAACCAATGTATTAGAGATGATG
AAGGAATGGAAAAATAACCCAGAAAGTGTTGAGGAAAACACAACAGAA
GAGAAGAA TAAGCCGTCCACTGAAGAAAAGTTGAAAAGTTACCAACCC
TGGGGAATGTCCCTGAGACCAATTACACTTCAGATGAAGAGGAGGAA
AAACAGTCTACTACTTATGAAAAGCAATTTCTGCAACCAGTAAGGAC
AACTTTGATATGTGCAGTTCAAGTTTTCACATCTAGCAA ACTCCTTCCA
GCTGAAATCAAGGGAGAAAATGGAGAAAACCCCTGTGACTCCAAGCCAA
GGAACAGCAACCAAGTACCCTGCTAAATCCGGCGCCCTGTCCAGAACC
AAAAAGAACTCtaagcggccgc