Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TSSK1 [1 - 367]

Enzyme description:- TSSK1 [1 - 367]

Clone number:- DU 34845

Source:- Recombinant

Expression system:- *E.coli*, co-expressed

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 68, 397.88 daltons
Average Mass 68, 442.10 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.52

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % 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:-
CHKtide [KKKVSRSGLYRSPSMPENLNRPR] Final concentration: 300 μM

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Clone Data Sheet

TSSK1 [1 - 367]

Protein	TSSK1 [1 - 367]
Clone number	DU 34845
Species	Human
Accession number	AY028964
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MDDAAVLKR** **RGYLLGINLGEGSYAKVKSAYSERLKFNVAIKIIDRKKAPADFLEKFL** **PREIEILAMLNHCSIIKTYEIFETSHGKVYIVMELAVQGDLLELIKTR** **GALHEDEARKKFHQLSLAIKYCHDLDVVHRDLKCDNLLLDKDFNIKLS** **DFSFSKRCLRDDSGRMALSKTFCGSPAYAAPEVLQGIPYQPKVYDIWS** **LGVILYIMVCGSMPYDDSNIKKMLRIQKEHRVNFPRSKHLTGECKDLI** **YHMLQPDVNRRLHIDEILSHCWMQPKARGSPSVAINKEGESSRGTEPL** **WTPEPGSDKKSATKLEPEGEAQPQAQPETKPEGTAMQMSRQSEILGFP** **SKPSTMETEEGPPQQPPETRAQ**
Native sequence	Amino acids M1 – Q367 of human TSSK1. 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) at residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX-6P

Nucleotide sequence of insert

ggatccATGGATGACGCTGCTGTCCTCAAGCGACGAGGCTACCTCCTG
GGGATAAATTTAGGAGAGGGCTCCTATGCAAAAGTAAAATCTGCTTAC
TCTGAGCGCCTGAAGTTCAATGTGGCGATCAAGATCATCGACCGCAAG
AAGGCCCCCGCAGACTTCTTGGAGAAATTCCTTCCCCGGGAAATTGAG
ATTCTGGCCATGTTAAACCACTGCTCCATCATTAAGACCTACGAGATC
TTTGAGACATCACATGGCAAGGTCTACATCGTCATGGAGCTCGCGGTC
CAGGGCGACCTCCTCGAGTTAATCAAAACCCGGGGAGCCCTGCATGAG
GACGAAGCTCGCAAGAAGTTCCACCAGCTTTCCTTGGCCATCAAGTAC
TGCCACGACCTGGACGTCGTCCACCGGGACCTCAAGTGTGACAACCTT
CTCCTTGACAAGGACTTCAACATCAAGCTGTCCGACTTCAGCTTCTCC
AAGCGCTGCCTGCGGGATGACAGTGGTCGAATGGCCTTAAGCAAGACC
TTCTGTGGGTCACCAGCGTATGCGGCCCCAGAGGTGCTGCAGGGCATT
CCCTACCAGCCCAAGGTGTACGACATCTGGAGCCTAGGCGTGATCCTC
TACATCATGGTCTGCGGCTCCATGCCCTACGACGACTCCAACATCAAG
AAGATGCTGCGTATCCAGAAGGAGCACCGCGTCAACTTCCCACGCTCC
AAGCACCTGACAGGCGAGTGCAAGGACCTCATCTACCACATGCTGCAG
CCCGACGTCAACCGGCGGCTCCACATCGACGAGATCCTCAGCCACTGC
TGGATGCAGCCCAAGGCACGGGGATCTCCCTCTGTGGCCATCAACAAG
GAGGGGGAGAGTTCCCGGGGAACTGAACCCTTGTGGACCCCCGAACCT
GGCTCTGACAAGAAGTCTGCCACCAAGCTGGAGCCTGAGGGAGAGGCA
CAGCCCCAGGCACAGCCTGAGACAAAACCCGAGGGGACAGCAATGCAA
ATGTCCAGGCAGTCGGAGATCCTGGGTTTCCCCAGCAAGCCGTCGACT
ATGGAGACAGAGGAAGGGCCCCCCCAACAGCCTCCAGAGACGCGGGCC
CAGtgagcggccgc