Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active TESK1 [1 – 345]

Enzyme description:- TESK1 [1 - 345]

Clone number:- DU 36111

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 64, 569.10 daltons
Average Mass 64, 610.57 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.64

Purity:- 85 %

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, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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

Substrate:-
Cofilin2 (1 – 166) [DU 427] Final concentration: 0.2 mg/ml

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Clone Data Sheet

TESK1 [1 - 345]

Protein	TESK [1 - 345]
Clone number	DU 36111
Species	Human
Accession number	NM_006285.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MAGERPPLRGPGPG PGEVPGEGPPGPGGTGGGPGRGRPSSYRALRSAVSSLARVDDFHCAEKI GAGFFSEVYKVRHRQSGQVMVLKMNKLPSNRGNTLREVQLMNRLRHPNI LRFMGVCVHQGQLHALTEYMNGGTLEQLLSSPEPLSWPVRLHLALDIAR GLRYLHSKGVFHRDLTSKNCLVRREDRGFTAVVGDFGLAEKIPVYREGA RKEPLAVVGSPYWMAPEVLRGELYDEKADVFAFGIVLCELIARVPADPD YLPRTEDFGLDVPAFRTLVGDDCPLPFLLLAIHCCNLEPSTRAPFTEIT QHLEWILEQLPEPAPLTRTALTHNQGSVARGGPSATL**
Native sequence	Amino acids M1 – L345 (end residue is S626) of human TESK1. 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 (LEVLFQGP) residues 221 - 229
Cloning sites	*Bg*III and *Not*1 sites of pGEX 6P-2

Nucleotide Sequence of insert

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTG
CAACCCACTCGACTTCTTTTGGAATATCTTGAAGAAAAATAT
GAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGA
AACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCT
TATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCC
ATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGT
TGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGT
AAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTA
CCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAA
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATG
TTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATG
TGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGT
ATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTT
GGTGGTGGCGACCATCCTCCAAAATCGGATCTGGAAGTTCTG
TTCCAGGGGCCCCTGGGATCTATGGCCGGGGAACGGCCCCCA
CTGCGGGGCCCTGGGCCCGGGCCTGGAGAGGTGCCGGGGGAG
GGGCCCCCGGGGCCGGGGGGCACGGGCGGAGGCCCGGGCCGG
GGCCGCCCCTCCTCCTACCGGGCTCTCCGCAGCGCCGTGTCT
AGCCTGGCGCGTGTGGACGATTTTCACTGCGCGGAGAAGATC
GGGGCCGGCTTCTTCTCTGAGGTCTACAAGGTTCGGCACCGA
CAGTCAGGGCAAGTCATGGTGCTGAAGATGAACAAGCTCCCC
AGTAACCGGGGCAACACACTACGGGAAGTGCAGCTGATGAAC
CGGCTCAGGCACCCCAACATCCTAAGGTTCATGGGAGTCTGT
GTGCACCAGGGACAGCTGCACGCTCTTACAGAGTATATGAAT
GGGGGGACATTGGAACAGCTGCTCAGCTCCCCTGAACCCCTA
TCCTGGCCGGTCAGGCTCCACCTGGCCCTGGACATTGCCCGA
GGCCTGCGGTACCTGCACTCCAAAGGTGTATTTCACCGCGAC
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TTCACCGCTGTCGTGGGTGACTTCGGGCTGGCCGAAAAGATT
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GAGCTGTATGATGAGAAGGCTGATGTCTTTGCCTTCGGGATT
GTCCTCTGTGAGCTCATCGCCCGAGTACCTGCAGACCCTGAC
TACCTACCACGCACTGAGGACTTTGGCCTGGATGTGCCTGCT
TTCCGAACTCTGGTGGGGGATGACTGCCCACTGCCTTTCTTG
CTCCTGGCCATCCACTGCTGCAACCTGGAACCCAGCACCCGT
GCCCCCTTCACCGAAATTACCCAGCACCTGGAATGGATCCTG
GAGCAGCTGCCTGAGCCAGCCCCACTCACCAGGACCGCCCTG
ACACACAATCAGGGCTCTGTTGCAAGAGGGGGTCCCTCTGCC
ACGCTTtag

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