

Division of Signal Transduction 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]

<u>Protein</u>	TESK [1 - 345]
<u>Clone number</u>	DU 36111
<u>Species</u>	Human
<u>Accession number</u>	NM_006285.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMAGERPPLRGPGPG PGEVPGEGPPGPGGTGGGPGRGRPSSYRALRSAVSSSLARVDDFHCAEKI GAGFFSEVYKVRHRQSGQVMVLKMNKLPSNRGNTLREVQLMNRLRHPNI LRFMGVCVHQQLHALTEYMNGGTLEQLLSSPEPLSWPVRLHLALDIAR GLRYLHSGGVFHRDLTSKNCLVRREDRGFTAVVGDFGLAEKIPVYREGA RKEPLAVVGSPYWMAPEVLRGELYDEKADVFAFGIVLCELIARVPADPD YLPRTEDFGLDVPAFRTL VGDDCPLPFLLLAIHCCNLEPSTRAPFTEIT QHLEWILEQLPEPAPLTRTALTHNQGSVARGGPSATL
<u>Native sequence</u>	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.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 229
<u>Cloning sites</u>	<i>B</i> gIII and <i>Not</i> I sites of pGEX 6P-2

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Nucleotide

Sequence of insert

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTG
CAACCCACTCGACTTCTTTTGAATATCTTGAAGAAAAATAT
GAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGA
AACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCT
TATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCC
ATCATACGTTATATAGCTGACAAGCACACATGTTGGGTGGT
TGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGT
AAAGACTTTGAACTCTCAAAGTTGATTTTCTTAGCAAGCTA
CCTGAAATGCTGAAAATGTTCTGAAGATCGTTTATGTCATAAA
ACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATG
TTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATG
TGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAACGT
ATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTT
GGTGGTGGCGACCATCCTCCAAAATCGGATCTGGAAGTTCTG
TTCCAGGGGCCCCCTGGGATCTATGGCCGGGAACGGCCCCCA
CTGCGGGGCCCTGGGCCCCGGCCTGGAGAGGTGCCGGGGGAG
GGGCCCCCGGGGCCGGGGGGCACGGGCGGAGGCCCGGGCCGG
GGCCGCCCCCTCCTCCTACCGGGCTCTCCGCAGCGCCGTGTCT
AGCCTGGCGCGTGTGGACGATTTTCACTGCGCGGAGAAGATC
GGGGCCGGCTTCTTCTCTGAGGTCTACAAGGTTCCGGCACC GA
CAGTCAGGGCAAGTCATGGTGCTGAAGATGAACAAGCTCCCC
AGTAACCGGGGCAACACACTACGGGAAGTGCAGCTGATGAAC
CGGCTCAGGCACCCCAACATCCTAAGGTTTATGGGAGTCTGT
GTGCACCAGGGACAGCTGCACGCTCTTACAGAGTATATGAAT
GGGGGGACATTGGAACAGCTGCTCAGCTCCCCCTGAACCCCTA
TCCTGGCCGGTCAGGCTCCACCTGGCCCTGGACATTGCCCGA
GGCCTGCGGTACCTGCACTCCAAAGGTGTATTTACCGCGAC
CTCACATCCAAGAACTGTCTAGTCCGACGGGAAGATCGAGGC
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CCTGTGTATAGGGAGGGGGCAAGGAAGGAGCCATTGGCCGTG
GTGGGCTCCCCATACTGGATGGCTCCAGAGGTGTTACGGGGT
GAGCTGTATGATGAGAAGGCTGATGTCTTTGCCTTCGGGATT
GTCCTCTGTGAGCTCATCGCCCCGAGTACCTGCAGACCCTGAC
TACCTACCACGCACTGAGGACTTTGGCCTGGATGTGCCTGCT
TTCCGAACTCTGGTGGGGGATGACTGCCCACTGCCTTTCTTG
CTCCTGGCCATCCACTGCTGCAACCTGGAACCCAGCACCCGT
GCCCCCTTACCCGAAATTACCCAGCACCTGGAATGGATCCTG
GAGCAGCTGCCTGAGCCAGCCCCACTCACCAGGACCGCCCTG
ACACACAATCAGGGCTCTGTTGCAAGAGGGGGTCCCTCTGCC
ACGCTTtag

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