

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TLK1 [1 – 766]

Enzyme description:- TLK1 [1 - 766]

Clone number:- DU 19922

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 113, 452.68 daltons

Average Mass 113, 523.81 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.36

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:-

Myelin Basic Protein (MBP)

Final concentration: 1 mg/ml

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Clone Data Sheet

TLK1 [1 - 766]

Protein TLK [1 - 766]

Clone number DU 19922

Species Human

Accession number NM_012290.4

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMSVQSSSGSLEGPP
SWSQLSTSPTPGSAAAARSLLNHTPPSGRPREGAMDELHSLDPRRQELL
EARFTGVASGSTGSTGSCSVGAKASTNNESNHSFGSLGSLSDKESETP
EKKQSESSRGRKRKAENQNESSQGKSI GGRGHKISDYFEYQGGNGSSPV
RGIPPAIRSPQNSHSHSTPSSSVRPNSPPTALAFGDHPVQPKQLSFK
IIQTDLTMLKLAALSNKIQDLEKKEGRIDDLLRANCDLRRQIDEQOKL
LEKYKERLNKCI SMSKLLIEKSTQEKLS SREKSMQDRRLRGHFTTVRH
GASFTEQWTDGFAFQNLVKQEQEWNQOREDIERQRKLLAKRKPPTANNS
QAPSTNSEPKQRKNKAVNGAENDPFVRPNLPOLLTLAEYHEQEEIFKLR
LGHLKKEEAEIQAELERLERVRNLHIRELKRINNEDNSQFKDHPTLNER
YLLLHLLGRGGFSEVYKAFDLYEQRYAAVKIHQLNKS WRDEKKNYHKH
ACREYRIHKELDHPRIVKLYDYFSLD TDFCTVLEYCEGNDLDFYLKQH
KLMSEKEARSIVMQIVNALRYLNEIKPPIIHYDLKPGNILLVDGTACGE
IKITDFGLSKIMDDDSYGVGMDLTSQGAGTYWYLPPECFVVGKEPPKI
SNKVDVWSVGVIFFQCLYGRKPF GHNQSQDILQENTILKATEVQFPVK
PVVSSEAKAFIRRC LAYRKEDRFDVHQ LANDPYLLPHMRRSNS SGNLHM
AGLTASPTPPSSSIITY

Native sequence Amino acids M1 – Y766 (end) of human TLK1.
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 (LEVLFOGP) residues 221 - 229

Cloning sites *Bg*III and *Not*I sites of pGEX 6P-1

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Nucleotide
Sequence of insert

agatctATGAGTGTCCAAAGTAGCAGTGGAAGTTTGGAGGGGCGCCAT
CTTGGTCCCAGCTCTCCACGTCTCCAACCCCGGGCTCGGCGGGCGGCGGC
CAGGTCCCTGCTGAATCACACGCCGCCATCCGGGAGGCCAGGGAAGGT
GCAATGGATGAGCTTCATAGTCTGGATCCAAGAAGGCAAGAGTTATTGG
AAGCTAGATTTACTGGAGTTGCAAGTGGGAGCACTGGAAGTACGGGCAG
TTGCAGTGTGGAGCTAAAGCCTCAACAAATAACGAAAGCTCTAATCAC
AGTTTTGGAAGCTTGGGATCTTTAAGTGACAAAGAATCAGAGACACCGG
AGAAGAAACAATCGGAATCATCCAGGGGAAGAAAGAGAAAAGCAGAAAA
CCAGAATGAAAGTAGTCAGGGAAAAAGTATTGGGGGACGTGGCCACAAA
ATTAGCGACTATTTTGAATACCAGGGTGGAAATGGCTCAAGTCCAGTAA
GAGGCATACCTCCTGCAATCCGTTCTCCTCAAAATTCACATTCACATTC
CACTCCTCCTCATCTGTTGACCGAATAGCCCTTCTCCTACTGCATTA
GCATTTGGGGACCACCCTATTGTACAACCAAAGCAATTATCCTTTAAAA
TTATTCAGACTGATCTCACAATGCTGAAATTAGCAGCATTAGAAAGTAA
TAAAATCCAGGACCTGGAAAAGAAGGAGGGACGTATAGATGATTTGCTC
AGGGCTAACTGTGATCTCAGACGGCAAATAGATGAACAACAAAATTAC
TTGAAAATACAAAGAACGATTAATAAGTGCATATCAATGAGCAAGAA
ACTTCTTATTGAAAAGAGTACACAAGAAAAGCTGTCAAGCAGAGAGAAG
AGTATGCAAGATCGATTACGCCTCGGGCACTTTACAACAGTTAGACATG
GCGCTTCATTTACTGAACAATGGACAGATGGTTTTGCATTTCAGAATCT
TGTGAAGCAACAAGAATGGGTGAATCAGCAAAGGGAAGATATTGAAAGG
CAAAGGAACTTCTAGCCAAACGCAAACCTCCCACAGCTAATAATTCTC
AGGCACCTCTACCAATTCTGAACCAAAACAAAGGAAAAACAAAGCAGT
CAATGGAGCAGAGAATGATCCCTTTGTTAGACCAATTTACCACAACCTG
TTGACTTTGGCAGAATATCATGAACAGGAAGAAATTTCAAACCTTAGAC
TAGGACATCTCAAAAAGGAAGAGGCAGAAATCCAGGCAGAACTTGAACG
TTTGGAAAGAGTCAGAAATCTTCACATACGTGAGCTGAAAAGAATAAAC
AATGAAGATAATTCACAGTTCAAAGATCACCAACATTAATGAAAGAT
ATTTATTACTTCATCTGCTTGGTAGAGGTGGCTTTAGTGAAGTGTATAA
GGCTTTTGACCTTTATGAACAAAGATATGCTGCTGTGAAGATACATCAG
CTTAATAAAAAGCTGGAGAGATGAGAAGAAAGAAAACCTACCACAAACATG
CCTGCAGAGAGTATAGAATACACAAAGAACTGGATCACCCAGAAATAGT
TAACTCTATGATTATTTCTCCTTGGATACAGATACGTTTTGTACAGTG
TTAGAATACTGTGAAGGCAATGACTTGGATTTCTATCTGAAGCAACACA
AGTTAATGTCAGAGAAAGAAGCTCGGTCTATTGTAATGCAGATTGTA
TGCCTAAGATATCTCAATGAGATCAAACCCCTATTATACATTATGAT
CTTAAGCCAGGAAACATCCTACTGGTAGATGGAACAGCATGTGGTGA
TCAAATCACTGATTTTGGTCTGTCCAAGATTATGGATGATGATAGCTA
TGGTGTAGATGGAATGGATCTAACTTCCCAGGGGGCAGGCACTTACTGG
TATTTACCTCCTGAGTGTTTTGTAGTTGGAAAAGAGCCACCAAGATTT
CCAACAAGGTTGATGTATGGTCGGTTGGAGTCATCTTCTTTCAGTGTCT
TTATGGTAGAAAGCCATTTGGTCACAATCAATCTCAACAAGACATTCTT
CAAGAAAATACAATATTTAAAGCCACAGAAGTCCAGTTCCTGTAAAAC
CGGTTGTAAGCAGTGAAGCCAAGGCATTTATAAGACGCTGTTTGGCATA
TCGAAAAGAAGATCGATTTGATGTGCACCAGCTGGCAAATGACCATAC
CTTCTCCCACACATGAGAAGATCAAATTTCTCAGGAAACCTACACATGG
CTGGGCTGACAGCATCCCTACACCCCTTCTTCAAGCATAATTACTTA

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