

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]

<u>Protein</u>	TLK [1 - 766]
<u>Clone number</u>	DU 19922
<u>Species</u>	Human
<u>Accession number</u>	NM_012290.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMSVQSSSGSLEGPP SWSQLSTSPTPGSAAAARSLLNHTPPSGRPREGAMDELHSLDPRRQELL EARFTGVASGSTGSTGSCSVGAKASTNNNESSNHSFGSLGSLSDKESETP EKKQSESSRGRKRKAENQNESSQGKSIGGRGHKISDYFEYQGGNGSSPV RGIPPAIRSPQNSHSHSTPSSSVRPNSPSPTALAFGDHPVQPKQLSFK IIQTDLTMLKLALESNKIQDLEKKEGRIDDLRANCDLRRQIDEQOKL LEKYKERLNKCISMSKKLLIEKSTQEKLSREKSMQDRLRLGHFTTVRH GASFTEQWTDGFAFQNLVKQEQEWNQOREDIERQRKLLAKRKPPTANNS QAPSTNSEPKQRKNKAVNGAENDPFVRPNLPOLLTLAEYHEQEEIFKLR LGHLKKEEAEIQAELERLERVRNLHIRELKRINNEDNSQFKDHPTLNER YLLLHLLGRGGFSEVYKAFDLYEQRYAAVKIHQLNKS WRDEKKENYHKH ACREYRIHKELDHPRIVKLYDYFSLD TDTFCTVLEYCEGNDLDFYLKQH KLMSEKEARSIVMQIVNALRYLNEIKPPIIH YDLKPGNILLVDGTACGE IKITDFGLSKIMDDDSYGV DGM DLTSQGAGTYWYLPPECFVVGKEPPKI SNKVDVWSVGVIFFQCLYGRKPF GHNQSQQDILQENTILKATEVQFPVK PVVSSEAKAFIRRC LAYRKEDRFDVHQ LANDPYLLPHMRRSNSSGNLHM AGLTASPTPPSSSIITY
<u>Native sequence</u>	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.
<u>Protease cleavage</u>	PreScission (LEVLFGQP) residues 221 - 229
<u>Cloning sites</u>	<i>Bgl</i> III and <i>Not</i> I sites of pGEX 6P-1

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Nucleotide

Sequence of insert

agatcctATGAGTGTCCAAAGTAGCAGTGGAAGTTTGGAGGGGCGCCAT
CTTGGTCCCAGCTCTCCACGTCTCCAACCCCGGGCTCGGCGGGCGGCGGC
CAGGTCCCTGCTGAATCACACGCCGCCATCCGGGAGGCCCAGGGAAGGT
GCAATGGATGAGCTTCATAGTCTGGATCCAAGAAGGCAAGAGTTATTGG
AAGCTAGATTTTACTGGAGTTGCAAGTGGGAGCACTGGAAGTACGGGCAG
TTGCAGTGTGGAGCTAAAGCCTCAACAAATAACGAAAGCTCTAATCAC
AGTTTTTGAAGCTTGGGATCTTTAAGTGACAAAGAATCAGAGACACCGG
AGAAGAAACAATCGGAATCATCCAGGGGAAGAAAGAGAAAAGCAGAAAA
CCAGAATGAAAGTAGTCAGGGAAAAAGTATTGGGGGACGTGGCCACAAA
ATTAGCGACTATTTTGAATACCAGGGTGGAAATGGCTCAAGTCCAGTAA
GAGGCATACCTCCTGCAATCCGTTCTCCTCAAAATTACATTCACATTC
CACTCCTTCCTCATCTGTTCGACCGAATAGCCCTTCTCCTACTGCATTA
GCATTTGGGGACCACCCTATTGTACAACCAAAGCAATTATCCTTTAAAA
TTATTCAGACTGATCTCACAATGCTGAAATTAGCAGCATTAGAAAGTAA
TAAAATCCAGGACCTGGAAAAGAAGGAGGGACGTATAGATGATTTGCTC
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GCGCTTCATTTACTGAACAATGGACAGATGGTTTTTGCATTTTCAGAATCT
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CCTGCAGAGAGTATAGAATACACAAAGAACTGGATCACCCCAGAATAGT
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TATTTACCTCCTGAGTGTTTTGTAGTTGGAAAAGAGCCACCAAAGATTT
CCAACAAGGTTGATGTATGGTCGGTTGGAGTCATCTTCTTTTCAGTGTCT
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CAAGAAAATACAATATTAAAAGCCACAGAAGTCCAGTTCCTGTAAAAC
CGGTTGTAAGCAGTGAAGCCAAGGCATTTATAAGACGCTGTTTGGCATA
TCGAAAAGAAGATCGATTTGATGTGCACCAGCTGGCAAATGACCCATAC
CTTCTCCCACACATGAGAAGATCAAATTCTTCAGGAAACCTACACATGG
CTGGGCTGACAGCATCCCCTACACCCCTTCTTCAAGCATAATTACTTA

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