

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TLK2 [1 - 750]

<u>Enzyme description:-</u>	TLK2 [1 – 750]
<u>Clone number:-</u>	DU 33104
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Calculated molecular mass:-</u>	
Monoisotopic	90, 199.48 daltons
Average Mass	90, 255.79 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	8.50
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.	
<u>Storage temperature:-</u>	-70 °C
<u>Assay Buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc	
<u>Substrate:-</u>	
Myelin Basic Protein (MBP)	Final concentration: 1 mg/ml

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Clone Data Sheet

TLK2 [1 - 750]

<u>Protein</u>	TLK2 [1 – 750]
<u>Clone Number</u>	DU 33104
<u>Species</u>	Human
<u>Accession number</u>	NM_006852.3
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	<p>MSYYHHHHHDYDIPTTENLYFQGMGSGIQRPSTSTSSLVAAAMMEEELH SLDPRRQELLEARFTGVGVSKGPLNSESSNQSLCSVGSLSKDKEVETPEK KONDQRNRKRKAEPYETSQGKGTPRGHKISDYFEFAGGSAPGTSPGRSV PPVARSSPQHSLSNPLPRRVEQPLYGLDGSAAKEATEEQSALPTLMSVM LAKPRLDTEQLAQRGAGLCFTFVSAQONSPTSSTGSGNTEHSCSSQKQIS IQHRQTQSDLTIEKISALENSKNSDLEKKEGRIDDLRANCDLRRQIDE QQKMLEKYKERLNRCVTMSKLLIEKSKQEKMACRDKSMQDRLRLGHFT TVRHGASFTEQWTDGYAFQNLIKQOERINSQREEIERQRKMLAKRKPPA MGQAPPATNEQKQKRSKTNGAENETLTLAEYHEQEEIFKLRLGHLKKEE AEIQAELELERLVRNLHIRELKRIHNEDNSQFKDHPTLNDRYLLHLLG RGGFSEVYKAFDLTEQRYVAVKIHQLNKNWRDEKKNENYHKHACREYRIH KELDHPRIVKLYDYFSLDTSFCTVLEYCEGNDLDFYLKQHKLMSEKEA RSIIMQIVNALKYLNEIKPPIIHVDLKPKNILLVNGTACGEIKITDFGL SKIMDDDSYNSVDGMELTSQGAGTYWYLPPECFVVGKEPPKISNKVDVW SVGVIIFYQCLYGRKPFQHNQSQDILQENTILKATEVQFPKPVVTPEA KAFIRRCLAYRKEDRIDVQQLACDPYLLPHIRKSVSTSSPAGAAIASTS GASNNSSSN</p>
<u>Native sequence</u>	<p>Amino acids M1 – N750 (end) of human TLK2. Residue M44 of fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.</p>
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Not1</i> sites of pFastBAC HTb

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Nucleotide sequence of insert

gcggccgctATGATGGAAGAATTGCATAGCCTGGACCCACGACGGCAGG
AATTATTGGAGGCCAGGTTTACTGGAGTAGGTGTTAGTAAGGGACCACT
TAATAGTGAGTCTTCCAACCAGAGCTTGTGCAGCGTCGGATCCTTGAGT
GATAAAGAAGTAGAGACTCCCGAGAAAAAGCAGAATGACCAGCGAAATC
GGAAAAGAAAAGCTGAACCATATGAAACTAGCCAAGGGAAAGGCACTCC
TAGGGGACATAAAATTAGTGATTACTTTGAGTTTGTCTGGGGGAAGCGCG
CCAGGAACCAGCCCTGGCAGAAGTGTTCCACCAGTTGCACGATCCTCAC
CGCAACATTCCTTATCCAATCCCTTACCGCGACGAGTAGAACAGCCCCT
CTATGGTTTAGATGGCAGTGCTGCAAAGGAGGCAACGGAGGAGCAGTCT
GCTCTGCCAACCCTCATGTCAGTGATGCTAGCAAACCTCGGCTTGACA
CAGAGCAGCTGGCGCAAAGGGGAGCTGGCCTCTGCTTCACTTTTGTTC
AGCTCAGCAAAACAGTCCCTCATCTACGGGATCTGGCAACACAGAGCAT
TCCTGCAGCTCCCAAAAACAGATCTCCATCCAGCACAGACAGACCCAGT
CCGACCTCACAATAGAAAAATATCTGCACTAGAAAACAGTAAGAATTC
TGACTTAGAGAAGAAGGAGGGAAAGAAATAGATGATTTATTAAGAGCCAAC
TGTGATTTGAGACGGCAGATTGATGAACAGCAAAGATGCTAGAGAAAT
ACAAGGAACGATTAATAGATGTGTGACAATGAGCAAGAACTCCTTAT
AGAAAAGTCAAAACAAGAGAAGATGGCGTGTAGAGATAAGAGCATGCAA
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TGAATGCTTTAAAGTACTTAAATGAAATAAAACCTCCCATCATACACTA
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CATCCTACAAGAGAATACGATTCTTAAAGCTACTGAAGTGCAGTTCCCG
CCAAAGCCAGTAGTAACACCTGAAGCAAAGGCGTTTATTCGACGATGCT
TGGCCTACCGAAAGGAGGACCGCATTGATGTCCAGCAGCTGGCCTGTGA
TCCCTACTTGTTGCCTCACATCCGAAAGTCAGTCTCTACAAGTAGCCCT
GCTGGAGCTGCTATTGCATCAACCTCTGGGGCGTCCAATAACAGTTCTT
CTAATtgagcggccgc

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