

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TANK Binding Kinase 1 [1 - 729]

<u>Enzyme description:-</u>	TBK1 [1 - 729]
<u>Clone number:-</u>	DU 12469
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	0.1 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	110, 395.45 daltons
Average Mass	110, 466.29 daltons
	[cysteines reduced, methionines have not been oxidised]
<u>Theoretical pI:-</u>	6.18
<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, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
	50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc
<u>Substrate:-</u>	
	[KKKKERLLDDRHDSGLDSMKDEE] Residues 21 to 41 of human IKappaB alpha (+ 2 added Lys at N terminus) Final concentration: 300 μ M
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

TANK Binding Kinase 1 [1 - 729]

Protein TBK1 [1 - 729]

Clone number DU 12469

Species Human

Accession number NM_013254

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMQSTSNHLW
LLSDILGQGATANVFRGRHKKTGDLFAIKVFNNISFLRPVDVQMRFE
VLKKNLHKNIVKLF AIEEETTTRHKVLIMEFCPCGSLYTVLEEPSNAY
GLPESEFLIVLRDVVGGMNLRENGIVHRDIKPGNIMRVI GEDGQSVY
KLTDFGAARELEDDEQFVSLYGTEEYLHPMYERAVLRKDHQKYGAT
VDLWSIGVTFYHAATGSLPFRPFEGPRRNKEVMYKIITGKPSGAI SGV
QKAENGPIDWSDMPVSCSLSRGLQVLLTPVLANILEADQEKCWGFDQ
FFAETSDILHRMVIHVFSLQOMTAHKIYIHSYNTATIFHEL VYKQTKI
ISSNQELIYEGRRLVLEPGRLAQHFPKTTEENPIFVVSREPLNTIGLI
YEKISLPKVHPRYDLGDASMAKAITGVVCYACRIASTLLLYQELMRK
GIRWLIELIKDDYNETVHKKTEVVITLDFCIRNIEKTVKVYEKLMKIN
LEAAELGEISDIHTKLLRLSSSQGTIETSLODIDSRLSPGGSLADAWA
HQEGTHPKDRNVEKLOVLLNCMTEIYYQFKDKAERRLAYNEEQIHKF
DKQKLYYHATKAMTHFTDECVKKYEAFLNKSEEWIRKMLHLRKQLLSL
TNQCFDIEEEVSKYQEYTNELQETLPQKMFTASSGIKHTMTP IYSSN
TLVEMTLGMKKLKEEMEGVVKELAENNHILERFGSLTMDGGLRNVDCI

Native sequence Amino acids M1 – L729 (end) of human TBK1.
Residue M232 of the fusion protein is equivalent to M1 of TBK1.
The GST tag is located at residues 1 - 220 of the fusion protein.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

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Cloning sites

*Bam*H1 of pFastBac GST 6P-1

Nucleotide sequence of insert

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ggatccATGCAGAGCACTTCTAATCATCTGTGGCTTTTATCTGATATT  
TTAGGCCAAGGAGCTACTGCAAATGTCTTTTCGTGGAAGACATAAGAAA  
ACTGGTGATTTATTTGCTATCAAAGTATTTAATAACATAAGCTTCCTT  
CGTCCAGTGGATGTTCAAATGAGAGAATTTGAAGTGTTGAAAAAACTC  
AATCACAAAAATATTGTCAAATTATTTGCTATTGAAGAGGAGACAACA  
ACAAGACATAAAGTACTTATTATGGAATTTTGTCCATGTGGGAGTTTA  
TACACTGTTTTAGAAGAACCTTCTAATGCCTATGGACTACCAGAATCT  
GAATTCCTAATTGTTTTGCGAGATGTGGTGGGTGGAATGAATCATCTA  
CGAGAGAATGGTATAGTGCACCGTGATATCAAGCCAGGAAATATCATG  
CGTGTTATAGGGGAAGATGGACAGTCTGTGTACAAACTCACAGATTTT  
GGTGCAGCTAGAGAATTAGAAGATGATGAGCAGTTTTGTTTCTCTGTAT  
GGCACAGAAGAATATTTGCACCCTGATATGTATGAGAGAGCAGTGCTA  
AGAAAAGATCATCAGAAGAAATATGGAGCAACAGTTGATCTTTGGAGC  
ATTGGGGTAACATTTTACCATGCAGCTACTGGATCACTGCCATTTAGA  
CCCTTTGAAGGGCCTCGTAGGAATAAAGAAGTGATGTATAAAATAATT  
ACAGGAAAGCCTTCTGGTGCAATATCTGGAGTACAGAAAGCAGAAAAT  
GGACCAATTGACTGGAGTGGAGACATGCCTGTTTCTTGCAGTCTTTCT  
CGGGGTCTTCAGGTTCTACTTACCCCTGTTCTTGCAAACATCCTTGAA  
GCAGATCAGGAAAAGTGTTGGGGTTTTGACCAGTTTTTTTGCAGAACT  
AGTGATATACTTCACCGAATGGTAATTCATGTTTTTTTCGCTACAACAA  
ATGACAGCTCATAAGATTTATATACATAGCTATAATACTGCTACTATA  
TTTCATGAACTGGTATATAAACAACCAAATTTATTTCTTCAAATCAA  
GAACTTATCTACGAAGGGCGACGCTTAGTCTTAGAACCTGGAAGGCTG  
GCACAACATTTCCCTAAACTACTGAGGAAAACCCTATATTTGTAGTA  
AGCCGGGAACCTCTGAATACCATAGGATTAATATATGAAAAAATTTCC  
CTCCCTAAAGTACATCCACGTTATGATTTAGACGGGGATGCTAGCATG  
GCTAAGGCAATAACAGGGGTTGTGTGTTATGCCTGCAGAATTGCCAGT  
ACCTTACTGCTTTATCAGGAATTAATGCGAAAGGGGATACGATGGCTG  
ATTGAATTAATTAAGATGATTACAATGAAACTGTTCCAAAAAGACA  
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GTGAAAGTATATGAAAAGTTGATGAAGATCAACCTGGAAGCGGCAGAG  
TTAGGTGAAATTTTCAGACATACACACCAAATTTGTTGAGACTTTCCAGT  
TCTCAGGGAACAATAGAAACCAGTCTTCAGGATATCGACAGCAGATTA  
TCTCCAGGTGGATCACTGGCAGACGCATGGGCACATCAAGAAGGCACT  
CATCCGAAAGACAGAAATGTAGAAAACTACAAGTCCTGTTAAATTGC  
ATGACAGAGATTTACTATCAGTTCAAAAAAGACAAAGCAGAACGTAGA  
TTAGCTTATAATGAAGAACAATCCACAAATTTGATAAGCAAAAAGCTG  
TATTACCATGCCACAAAAGCTATGACGCACTTTACAGATGAATGTGTT  
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ATGCTTCATCTTAGGAAACAGTTATTATCGCTGACTAATCAGTGTTTTT  
GATATTGAAGAAGAAGTATCAAAATATCAAGAATATACTAATGAGTTA  
CAAGAACTCTGCCTCAGAAAATGTTTACAGCTTCCAGTGGAATCAAA  
CATAACCATGACCCCAATTTATCCAAGTTCCTAACACATTAGTAGAAATG  
ACTCTTGGTATGAAGAAATTAAGGAAGAGATGGAAGGGGTGGTTAAA  
GAACTTGCTGAAAATAACCACATTTTAGAAAGGTTTGGCTCTTTAACC
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ATGGATGGTGGCCTTCGCAACGTTGACTGTCTTtagggatcc