

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active TTBK1 [1 - 1321]**

**Enzyme description:-** TTBK1 [1 - 1321]

**Clone number:-** DU 34496

**Source:-** Recombinant

**Expression system:-** *E.coli*, co-expressed

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 169, 456.38 daltons

Average Mass 169, 561.26 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.50

**Purity:-** >80 %

**Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,  
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-** -70 °C

**Assay buffer:-**

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

**Substrate:-**

CHKtide [KKKVSRSGLYRSPSPENLNRPR]

Final concentration: 300  $\mu$ M

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**Clone Data Sheet**

**TTBK1 [1 - 1321]**

**Protein** TTBK1 [1 - 1321]

**Clone number** DU 34496

**Species** Human

**Accession number** NM\_032538.1

**Tags** N-terminal GST

**Bacterially  
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE  
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMQCLAAALK  
**DETNMSGGGEQADILPANYVVKDRWKVLKKIGGGGFGEIYEAMDLLTR**  
**ENVALKVESAQQPKQVLKMEVAVLKKLQKGDHVCRFIGCGRNEKFNYV**  
**VMQLQGRNLADLRRSQPRGTFTLSTTLRLGKQILESIEAIHSVGFLHR**  
**DIKPSNFAMGRLPSTYRKCYMLDFGLARQYTNNTGDVPRPRNVAGFRG**  
**TVRYASVNAHKNREMGRHDDLWSLFYMLVEFAVGQLPWRKIKDKEQVG**  
**MIKEYEHRMLLKHPSEFHLFLDHIASLDYFTKPDYQLIMSVFENSM**  
**KERGIAENEAFDWEKAGTDALLSTSTSTPPQONTRQTAAMFGVVNVTP**  
**VPGDLLRENTEDVLQGEHLSQENAPPILPGRPSEGLGPSPLHVPHPG**  
**GPEAEVWEETDVNRNKLRLINIGKSPCVEEEQSRGMGVPSSPVRAPPDS**  
**PPTPVRSRLYRRVNSPESERLSTADGRVELPERRSRMDLPGSPSRQAC**  
**SSQPAQMLSVDTGADRQASGRMDVSASVEQEALSNAFRSVPLAEED**  
**FDSKEWVIIDKETELKDFPPGAEPSTSGTTDEEPEELRPLPEEGEERR**  
**RLGAEPTVRPRGRSMQALAEEDLQHLPPQPLPPQLSQGDGRSETSOPP**  
**TPGSPSHSPLHSGPRRRRESDPTGPQRQVFSVAPPFEVNGLPRAVPL**  
**SLPYQDFKRDLSDYRERARLLNRVRRVGFSHMLLTPQVPLAPVQPQA**  
**NGKEEEAAAAVA**  
**LGEVLGPRSGSSSEGSESTDRSQEGAPSTLLADDQKESRGRASMADG**  
**DLEPEEGSKTLVLVSPGDMKKSPVTAELAPDPDLGTLAALTPQHERPQ**  
**PTGSQLDVSEPGTLSSVLKSEPKPPGPGAGLGAGTVTTGVGGVAVTSS**  
**PFTKVERTFVHIAEKTHLNVMSGGQALRSEEF SAGGELGLELASDGG**  
**AVEGARAPLENGLALSGLNGAEIEGSALSGAPRETPSEMATNSLPNG**  
**PALADGPAPVSPLEPSPEKVATISPRRHAMPGSRPRSRI PVLLSEEDT**  
**GSEPSGSLSAKERWSKRARPQODLARLVMEKRQGRLLLLRLASGASSSS**  
**SEEQRRASETLSGTGSEEDTPASEPAAALPRKSGRAAATRSRI PRPIG**  
**LRMPMPVAAQQOPASRSHGAAPALDTAITSRLQLQTPPGSATAADLRPK**  
**QPPGRGLGPGRAQAGARPPAPRSRPLPASTSAARNASASPRSQSLRR**

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**ESPSPSHQARPGVPPPRGVPPARAQPDGTPSPGGSKKGPRGKLAQORA  
TTKGRAGGAEGRAGAR**

### **Native sequence**

Amino acids M1 – R1321 (end) of human TTBK1.

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 site (LEVLFOGP) at residues 221 - 228

### **Cloning sites**

*Bam*H1 and *Not*I sites of pGEX-6P1

### **Nucleotide sequence of insert**

```
ggatccATGCAGTGCCTAGCGGCAGCTCTTAAGGACGAAACCAACATG
AGTGGGGGAGGGGAGCAGGCCGACATCCTGCCGGCCAACTACGTGGTC
AAAGATCGCTGGAAGGTGCTGAAAAAGATCGGGGGCGGGGGCTTCGGT
GAGATCTATGAGGCCATGGACCTGCTGACCAGGGAGAACGTGGCTCTC
AAGGTGGAGTCAGCCCAACAGCCTAAGCAGGTCCTCAAGATGGAGGTG
GCTGTGCTCAAAAAGCTTCAAGGCAAGGACCACGTGTGCAGGTTTCATT
GGCTGTGGCCGTAACGAGAAGTTTAACTATGTGGTGATGCAGCTCCAG
GGCCGGAACCTGGCTGACCTGCGCCGACCCAGCCAAGGGGCACCTTC
ACGTTGAGTACCACACTGCGCCTGGGCAAGCAGATCCTGGAGTCCATT
GAAGCCATTCACCTCCGTGGGCTTCCCTGCACCGTGACATCAAGCCGTCC
AACTTTGCCATGGGCCGGCTGCCCTCGACCTACAGGAAGTGCTACATG
TTGGACTTTGGGCTAGCCAGGCAGTACACTAACACCACTGGGGACGTC
CGGCCTCCTCGGAATGTGGCCGGGTTCCGGGGGACTGTCCGCTACGCC
TCGGTCAATGCTCACAAGAACC GGGAGATGGGCCGCCATGATGACCTG
TGGTCCCTCTTCTACATGCTGGTGGAGTTTGCTGTGGGCCAGCTGCC
TGGAGGAAGATCAAAGACAAGGAGCAGGTGGGGATGATCAAAGAGAAA
TACGAGCACCCGATGCTACTGAAGCACATGCCATCCGAGTTTCATCTC
TTCTGGACCACATTGCCAGCCTCGACTACTTCACCAAGCCCATTAC
CAGTTGATCATGTCAGTGTTTGAGAACAGCATGAAGGAGCGGGGCATT
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GCAGCCATGTTTGGAGTGGTCAATGTGACACCAGTGCCAGGGGATCTG
CTGAGGGAAAATACCGAAGACGTGCTCCAGGGGGAACATCTGTCCGAC
CAGGAAAACGCTCCACCAATCCTGCCTGGAAGACCATCTGAGGGCCTC
GGACCTAGTCCACACCTGGTGCCACATCCTGGAGGACCAGAAGCAGAA
GTGTGGGAGGAAACCGACGTGAACCGAAATAAGCTGAGGATCAACATT
GGCAAAAGCCCTTGCGTGGAGGAAGAGCAGTCCAGGGGAATGGGTGTG
CCAAGCTCCCCTGTCAGAGCTCCCCCTGATAGCCCAACCACACCCGTG
CGCTCCCTGCGATACAGGAGAGTCAATTCTCCTGAAAGTGAGCGACTC
TCTACAGCAGACGGAAGGGTGGAACTGCCAGAGCGGCGCAGCCGAATG
GATCTCCCTGGCAGTCCATCACGACAGGCCTGTTCTAGTCAGCCTGCT
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## *Division of Signal Transduction Therapy*

CAGATGCTGTCCGTGGACACTGGTCACGCCGATAGGCAGGCTTCTGGC  
AGAATGGACGTGAGCGCCTCCGTGCGAACAGGAGGCACTGTCAAACGCC  
TTCAGAAGCGTGCCACTCGCCGAAGAGGAAGACTTTGATAGCAAGGAG  
TGGGTGATCATTGACAAGGAAACCGAGCTGAAAGATTTCCACCAGGA  
GCAGAGCCATCTACAAGTGGAACTACCGATGAGGAACCTGAGGAACTG  
CGGCCACTCCCAGAGGAAGGAGAGGAACGAAGGAGACTGGGTGCAGAG  
CCTACAGTGCGCCCACGAGGCAGGAGCATGCAGGCACTCGCCGAGGAA  
GACCTGCAGCACCTCCCTCCACAGCCACTCCCACCTCAGCTGTCCAG  
GGAGATGGACGCTCAGAGACCAGCCAGCCACCCACACCCGGTTCACCT  
AGCCACTCCCCACTGCATTCAGGACCTCGGCCACGGCGCCGAGAGTCC  
GACCCAACCGGACCACAGCGACAGGTGTTCTCCGTGCTCCTCCATTT  
GAGGTCAATGGACTGCCAAGGGCAGTCCCTCTCAGCCTGCCATAACCAG  
GATTTCAAGAGAGACCTGTCCGATTATAGAGAGCGAGCACGACTGCTC  
AACCGGGTGAGGAGAGTCGGATTTTCTCATATGCTGCTCACAACCTCCT  
CAGGTGCCACTGGCACCCGTCCAGCCTCAGGCCAATGGGAAAGAGGAG  
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AGGAGCCAGGAGGGAGCTCCAAGCACCCCTGCTCGCAGACGATCAGAAG  
GAGTCCCGAGGCAGGGCCTCTATGGCTGACGGAGATCTGGAGCCTGAA  
GAGGGGTCTAAAACCTCTCGTGCTGGTCAGTCCAGGCGACATGAAGAAA  
TCACCCGTGACCCGAGAGCTCGCACCCGACCCTGATCTGGGAACTCTC  
GCTGCACTGACCCCACAGCACGAGAGGCCACAGCCAACAGGTAGTCAG  
CTGGACGTGAGCGAACCTGGCACTCTCTCTAGTGCTCTGAAGTCCGAG  
CCAAAACCACCTGGACCTGGTGCTGGACTGGGAGCAGGGACTGTGACC  
ACAGGAGTCGGGGGTGTGGCTGTACATCAAGCCCTTTCACTAAGGTG  
GAACGCACCTTTGTCCACATCGCCGAGAAAACCCATCTGAACGTGATG  
TCCTCTGGCGGACAGGCTCTCAGGAGCGAAGAGTTTTTCCGAGGAGGT  
GAACTCGGACTGGAGCTCGCTTCCGACGGAGGAGCAGTGGAAGAGGGA  
GCTAGAGCACCCCTGGAGAACGGGCTGGCCCTCTCTGGACTCAATGGG  
GCTGAAATTGAGGGCAGTGCCCTGTGAGGAGCTCCACGAGAAACACCT  
TCCGAGATGGCTACTAACTCTCTCCCAAATGGACCCGCCCTGGCTGAT  
GGACCTGCCCCAGTGAGCCACTGGAACCATCCCCTGAGAAGGTGCGCA  
ACCATCAGCCCTCGGCGCCATGCTATGCCAGGCTCTAGACCCCGGAGT  
CGCATTCTGTGCTGCTCAGTGAAGAGGACACAGGGTCAGAACCATCT  
GGTAGTCTGTGTCAGCCAAGGAGCGGTGGTCTAAACGAGCAAGGCCCTCAG  
CAGGATCTCGCCGCCTGGTGATGGAGAAGAGGCAGGGAAGACTGCTC  
CTGAGGCTGGCTAGCGGGGCAAGTTCAAGCTCCTCTGAAGAGCAGCGA  
AGGGCTTCAGAGACACTGAGCGGTACTGGCTCCGAAGAGGACACACCT  
GCATCCGAGCCAGCCGCTGCACTGCCACGAAAATCTGGCCGAGCCGCT  
GCAACTAGAAGTAGAATCCCCGGCCATTGGACTGCGCATGCCTATG  
CCAGTGGCCGCTCAGCAGCCTGCTAGCAGATCCCACGGAGCAGCACCA

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GCACTGGACACTGCCATCACCTCTCGACTGCAGCTCCAGACCCCACCA  
GGCAGTGCCACAGCTGCAGATCTGAGGCCAAAGCAGCCTCCAGGACGA  
GGACTCGGTCCAGGAAGGGCACAGGCAGGAGCTAGACCCCCTGCACCT  
CGGTCCCCACGACTGCCAGCATCTACAAGTGCCGCTAGAAACGCATCA  
GCCAGCCCACGGTCCCAGTCTCTGAGTAGACGGGAGTCACCCAGCCCT  
TCCCATCAGGCACGACCAGGAGTGCCACCACCTCGAGGTGTCCCACCC  
GCTCGAGCACAGCCAGACGGAACCCCATCTCCAGGAGGTAGTAAGAAA  
GGTCCCAGGGCAAGCTGCAGGCCAGAGGGCAACTACTAAAGGTCGG  
GCAGGAGGTGCTGAAGGAAGGGCAGGGGCAAGGtgagcggccgc