

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TTK [1 - 857]

<u>Enzyme description:-</u>	TTK [1 – 857]
<u>Clone number:-</u>	DU 8837
<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>Expression level:-</u>	1 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	100, 380.51 daltons
Average Mass	100, 443.89 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	8.10
<u>Purity:-</u>	75 %
<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 [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay Buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc	
<u>Substrate:-</u>	
RSRSRSRSRSRSRSR	residues 204 – 218 of human ASF-1/SF-2
Final concentration:	300 μM
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

TTK [1 - 857]

Protein TTK [1 – 857]

Clone Number DU 8837

Species Human

Accession number NM_003318

Tags N-terminal His(6)

Baculovirus expressed protein

MSYYHHHHHDYDIPTTENLYFQGAMGSMESDLSGRELTIDSIMNKVR
DIKNKFKNEDLTDELSLNKISADTTDNSGTVNQIMMANNPEDWLSLLL
KLEKNSVPLSDALLNKLIGRYSQAIEALPPDKYGONESFARIQVRFAEL
KAIQEPDDARDYFQMARANCKKFAFVHISFAQFELSQGNVKKSKQLLOK
AVERGAVPLEMLEIALRNLNLQKKQLLSEEEKNLSASTVLTAEFSFG
SLGHLQNRNNSCDSRGQTTKARFLYGENMPPQDAEIGYRNSLRQTNKTK
QSCPFGRVPVNLNLPDCDVKTDDSVVPCFMKRQTSRSECRDLVVPGSK
PSGNDSCELRNLKSVQNSHFKEPLVSDKSSSELIITDSITLKNKTESSL
LAKLEETKEYQEPEVPESNQQWQSKRKSECINQNPAASSNHWQIPELA
RKVNTEQKHTTFEQPVFSVKQSPPISTSKWFDPKSICKTPSSNTLDDY
MSCFRTPVVKNDFPACQLSTPYGQPACFQQQHQILATPLQNLQVLAS
SSANECISVKGRIYSILKQIGSGGSSKVFQVLNEKKQIYAIKYVNLEEA
DNQTLDSYRNEIAYLNKLQHQSDKIIRLYDYEITDQYIYMVMCEGNIDL
NSWLKKKKSIDPWERKSYWKNMLEAVHTIHQHGIVHSDLKPANFLIVDG
MLKLIDFGIANQMPDPTTSVVKDSQVGTVNYPPEAIKDMSSSRENGKS
KSKISPKSDVWSLGCILYMYTYGKTPFQOIINQISKLHAIIDPNHEIEF
PDIPEKDLQDVLKCLKRPKQORISIPELLAHPYVQIQTHPVNQMAKGT
TEEMKYVLGQLVGLNSPNSILKAAKTLYEYSGGESHNSSSSKTFEKKR
GKK

Native sequence Amino acids M1 – K857 (end) of human TTK.
Residue M29 of fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Bam*H1 and *Not*1 site of pFastBAC HTb

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Nucleotide sequence of insert

ggatccATGGAATCCGAGGATTTAAGTGGCAGAGAATTGACAATTGATT
CCATAATGAACAAAGTGAGAGACATTAATAAATAAGTTTAAAAATGAAGA
CCTTACTGATGAACCTAAGCTTGAATAAAATTTCTGCTGATACTACAGAT
AACTCGGGAAGTGTAAACCAAAATTTATGATGATGGCAAACAACCCAGAGG
ACTGGTTGAGTTTGTGCTCAAACCTAGAGAAAAACAGTGTTCGGCTAAG
TGATGCTCTTTTAAATAAAATTGATTGGTCGTTACAGTCAAGCAATTGAA
GCGCTTCCCCCAGATAAATATGGCCAAAATGAGAGTTTTGCTAGAATTC
AAGTGAGATTTGCTGAATTAAGCTATTCAAGAGCCAGATGATGCACG
TGACTACTTTCAAATGGCCAGAGCAAACCTGCAAGAAATTTGCTTTTGT
CATATATCTTTTGCACAATTTGAACTGTCACAAGGTAATGTCAAAAAA
GTAAACAACCTTCTTCAAAAAGCTGTAGAACGTGGAGCAGTACCACTAGA
AATGCTGGAAATTGCCCTGCGGAATTTAAACCTCAAAAAAAGCAGCTG
CTTTCAGAGGAGGAAAAGAAGATTTATCAGCATCTACGGTATTAAGT
CCCAAGAATCATTTTCCGGTTCACCTGGGCATTTACAGAATAGGAACAA
CAGTTGTGATTCCAGAGGACAGACTACTAAAGCCAGGTTTTTATATGGA
GAGAACATGCCACCACAAGATGCAGAAATAGGTTACCGGAATTCATTGA
GACAACTAACAAAACCTAAACAGTCAATGCCATTTGGAAGAGTCCAGT
TAACCTTCTAAATAGCCAGATTGTGATGTGAAGACAGATGATTCAGTT
GTACCTTGTTTTATGAAAAGACAAACCTCTAGATCAGAATGCCGAGATT
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GATGAAAAGAGTTCTGAACCTTATTACTGATTCAATAACCCTGAAGA
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TCAAGAACCAGAGGTTCCAGAGAGTAACCAGAAACAGTGGCAATCTAAG
AGAAAGTCAGAGTGTATTAACCAGAATCCTGCTGCATCTTCAAATCACT
GGCAGATTCGGGAGTTAGCCCGAAAAGTTAATACAGAGCAGAAACATAC
CACTTTTGGAGCAACCTGTCTTTTTCAGTTTCAAAACAGTACCACCAATA
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GCCTGTTTCCAGCAGCAACAGCATCAAATACTTGCCACTCCACTTCAA
ATTTACAGTTTTTAGCATCTTCTTCAGCAAATGAATGCATTTTCGGTTAA
AGGAAGAATTTATTCATTTTAAAGCAGATAGGAAGTGGAGGTTCAAGC
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CGAAATAGCTTATTTGAATAAACTACAACAACACAGTGATAAGATCATC
CGACTTTATGATTATGAAATCACGGACCAGTACATCTACATGGTAATGG
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TGATCCATGGGAACGCAAGAGTTACTGGAAAAATATGTTAGAGGCAGTT
CACACAATCCATCAACATGGCATTGTTACAGTGATCTTAAACCAGCTA
ACTTTCTGATAGTTGATGGAATGCTAAAGCTAATTGATTTTGGGATTGC
AAACCAAATGCAACCAGATAACAACAAGTGTGTTAAAGATTCTCAGGTT
GGCACAGTTAATTATATGCCACCAGAAGCAATCAAAGATATGTCTTCCT
CCAGAGAGAATGGGAAATCTAAGTCAAAGATAAGCCCCAAAAGTGATGT
TTGGTCTTAGGATGTATTTTGTACTATATGACTTACGGGAAAACACCA
TTTCAGCAGATAATTAATCAGATTTCTAAATTACATGCCATAATTGATC
CTAATCATGAAATTGAATTTCCCGATATTCCAGAGAAAGATCTTCAAGA

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TGTGTTAAAGTGTGTTTAAAAAGGGACCCAAAACAGAGGATATCCATT
CCTGAGCTCCTGGCTCATCCCTATGTTCAAATTCAACTCATCCAGTTA
ACCAAATGGCCAAGGGAACCACTGAAGAAATGAAATATGTTCTGGGCCA
ACTTGTGGTCTGAATTCTCCTAACTCCATTTTGAAAGCTGCTAAACT
TTATATGAACACTATAGTGGTGGTGAAGTCATAATTCTTCATCCTCCA
AGACTTTTGAAAAAAAAAGGGGAAAAAAAtgagcggccgc