

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TTBK2 [1 – 443] Disease Variant Two

Disease Variant Two = one base insertion of an adenosine in exon 13 at nucleotide 1329 (codons 444), which creates a premature stop site (TGA) at codon 450.

Enzyme description:- TTBK2 [1 – 443] disease variant two

Clone number:- DU 34607

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 78, 141.03 daltons

Average Mass 78, 190.74 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.27

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, 0.2 mM PMSF, 1 mM Benzamidine

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 μ M

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Clone Data Sheet

TTBK2 [1 – 443] Disease Variant Two

Disease Variant Two = one base insertion of an adenosine in exon 13 at nucleotide 1329 (codons 444), which creates a premature stop site (TGA) at codon 450.

Protein TTBK2 [1 - 443]

Clone number DU 34607

Species Human

Accession number BC071556

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVCLTQSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSR IAYSKDFETLKVDFLSKLPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVL
QGPLGSPGIPGSTRAAAMSGGGEQPDILSVGILVKERWKVLRKIG
GGGFGEIYDALDMLTRENVALKVESAOQPKQVLKMEVAVLKKLQ
KDHVCRFIGCGRNDRFNYYVMQLQGRNLADLRRSQRGTFITST
LRLGRQILESIESIHSVGLHRDIKPSNFAMGRFPSTCRKCYMLD
FGLARQFTNSCGDVRPPRAVAGFRGTVRYASINAHNRNEMGRHDD
LWSLFYMLVEFVVGQLPWRKIKDKEQVGSIKERYDHRLMLKHLPP
EFSIFLDHISSLDYFTKPDYQLLTSVFDNSIKTFGVIESDPFDWE
KTGNDGSLTTTTTSTTPQLHTRLTPAAIGIANATPIPGDLLRENT
DEVFPDEQLSDGENGIPVGVSPDKLPGLGHPRPQEKDVWEEMDA
NKNKIKLGICKAAATEEENSHGQANGLLNAPSLGSPIRVRSEITQP
DRDIPLVRKL**TFHSQL**

Native sequence Amino acids M1 – L443 of human TTBK2, plus additional 6 amino acids [**TFHSQL**] due to disease variant [Full length protein ends at residue L690]
Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *NotI* sites of pGEX-6P-1

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**Nucleotide
sequence of insert**

gcggccgcgATGAGTGGGGGAGGAGAGCAGCCGATATCCTGAGT
GTTGGAATCCTAGTGAAAGAAAGATGGAAAGTGTTGAGAAAGATT
GGGGTGGGGGCTTTGGAGAAATTTACGATGCCTTGGACATGCTC
ACCAGGGAAAATGTTGCACTGAAGGTGGAATCAGCTCAACAACCA
AAACAAGTTCTGAAAATGGAAGTTGCTGTTTTGAAAAAGCTGCAA
GGGAAAGACCATGTTTGTAGATTTATTGGCTGTGGGAGGAATGAT
CGATTCAACTATGTGGTCATGCAGTTGCAGGGTCGGAATCTGGCA
GATCTTCGCCGTAGCCAGTCCCGAGGCACATTCACCATTAGTACC
ACTCTCCGGCTGGGTAGACAGATTTTGGAGTCTATTGAAAGCATT
CATTCTGTGGGATTCTTGCATCGAGACATCAAACCGTCGAACTTC
GCTATGGGTTCGCTTTCCTAGTACATGTAGGAAATGTTACATGCTT
GATTTTGGCTTGGCTCGACAATTTACCAATTCCTGTGGTGACGTC
AGACCACCTCGAGCTGTGGCAGGTTTTTCGAGGGACAGTTCGTTAT
GCATCAATCAACGCACATCGGAACAGGGAAATGGGAAGACATGAT
GACCTTTGGTCCCTTATTCTACATGTTGGTGGAGTTTTGTGGTTGGT
CAGCTGCCCTGGAGAAAAATAAAGGACAAGGAGCAAGTAGGCTCT
ATTAAGGAGAGATATGACCACAGGCTCATGTTGAAACATCTCCCT
CCAGAATTCAGCATCTTTCTAGACCATATCTCTTCTTTGGATTAT
TTTACAAAACCAGACTACCAGCTTCTTACATCCGTGTTTGACAAT
AGCATCAAGACTTTTGGAGTAATTGAGAGTGACCCTTTTACTGG
GAGAAGACTGGAAATGATGGCTCCCTAACAACCACCACTACTTCT
ACCACCCCTCAGTTGCACACTCGCTTGACCCCTGCTGCAATTGGA
ATTGCCAATGCTACTCCCATCCCTGGAGACTTGCTTCGAGAAAAT
ACAGATGAGGTATTTCCAGATGAACAGCTTAGCGATGGAGAAAAT
GGCATCCCTGTTGGTGTGTACCAGATAAATTGCCTGGATCTCTG
GGACACCCCGTCCCCAGGAGAAGGATGTTTGGGAAGAGATGGAT
GCCAACAAAAACAAGATAAAGCTTGGAAATTTGTAAGGCTGCTACT
GAAGAGGAGAACAGCCATGGCCAGGCAAATGGTCTTCTCAATGCT
CCAAGCCTTGGGTCACCAATTCGTGTCCGCTCAGAGATTACTCAG
CCAGACAGAGATATTCACCTGGTGCGAAAGTTA**ACATTCCATTCA
CAGCTT**tgagttagcggccgc