

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TTBK2 [1 – 428] Disease Variant One

Disease Variant One = Frameshift deletion of two base (GA) in exon 13, nucleotides 1284-1285 (codons 428, 429), which creates a premature stop site (TGA) at codon 449.

Enzyme description:- TTBK2 [1 – 428]

Clone number:- DU 34606

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 77,949.81 daltons

Average Mass 77,999.38 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.91

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 – 428] Disease Variant One

Disease Variant One = Frameshift deletion of two base (GA) in exon 13, nucleotides 1284-1285 (codons 428, 429), which creates a premature stop site (TGA) at codon 449.

Protein TTBK2 [1 - 428]

Clone number DU 34606

Species Human

Accession number BC071556

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
FQGPLGSPGIPGSTRAAAMSGGGEQPDILSVGILVKERWKVLRKIG
GGGFGEIYDALDMLTRENVALKVESAAQPKQVLKMEVAVLKKLQG
KDHVCRFIGCGRNDRFNYYVMQLQGRNLADLRRSQSRGTFTISTT
LRLGRQILESIESIHSVGFLLHRDIKPSNFAMGRFPSTCRKCYMLD
FGLARQFTNSCGDVRPPRAVAGFRGTVRYASINAHNRNEMGRHDD
LWSLFYMLVEFVVGQLPWRKIKDKEQVGSIKERYDHRLMLKHLPP
EFSIFLDHISSLDYFTKPDYQLLTSVFDNSIKTFGVIESDPFDWE
KTGNDGSLTTTTTSTTPQLHTRLTPAAIGIANATPIPGDLLRENT
DEVFPDEQLSDGENGIPVGVSPDKLPGLGHPRPQEKDVWEEMDA
NKNKIKLGICKAAATEEENSHGQANGLLNAPSLGSPIRVRS**DYSAR**
QRYSTGAKVTFHSQL

Native sequence Amino acids M1 – S428 of human TTBK2, plus additional 20 amino acids [**DYSARQRYSTGAKVTFHSQL**] 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

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Cloning sites

NotI sites of pGEX-6P-1

Nucleotide sequence of insert

gcggccgcgATGAGTGGGGGAGGAGAGCAGCCGGATATCCTGAGT
GTTGGAATCCTAGTGAAAGAAAGATGGAAAGTGTTGAGAAAGATT
GGGGGTGGGGGCTTTGGAGAAATTTACGATGCCTTGGACATGCTC
ACCAGGGAAAATGTTGCACTGAAGGTGGAATCAGCTCAACAACCA
AAACAAGTTCTGAAAATGGAAGTTGCTGTTTTTAAAAAGCTGCAA
GGGAAAGACCATGTTTGTAGATTTATTGGCTGTGGGAGGAATGAT
CGATTCAACTATGTGGTCATGCAGTTGCAGGGTCGGAATCTGGCA
GATCTTCGCCGTAGCCAGTCCCGAGGCACATTCACCATTAGTACC
ACTCTCCGGCTGGGTAGACAGATTTTGGAGTCTATTGAAAGCATT
CATTCTGTGGGATTCTTGCATCGAGACATCAAACCGTCGAACTTC
GCTATGGGTCGCTTTCCTAGTACATGTAGGAAATGTTACATGCTT
GATTTTGGCTTGGCTCGACAATTTACCAATTCCTGTGGTGACGTC
AGACCACCTCGAGCTGTGGCAGGTTTTTCGAGGGACAGTTCGTTAT
GCATCAATCAACGCACATCGGAACAGGGAAATGGGAAGACATGAT
GACCTTTGGTCCTTATTCTACATGTTGGTGGAGTTTGTGGTTGGT
CAGCTGCCCTGGAGAAAAATAAAGGACAAGGAGCAAGTAGGCTCT
ATTAAGGAGAGATATGACCACAGGCTCATGTTGAAACATCTCCCT
CCAGAATTCAGCATCTTTCTAGACCATATCTCTTCTTTGGATTAT
TTTACAAAACCAGACTACCAGCTTCTTACATCCGTGTTTGACAAT
AGCATCAAGACTTTTGGAGTAATTGAGAGTGACCCTTTTGACTGG
GAGAAGACTGGAAATGATGGCTCCCTAACAACCACCACTACTTCT
ACCACCCCTCAGTTGCACACTCGCTTGACCCCTGCTGCAATTGGA
ATTGCCAATGCTACTCCCATCCCTGGAGACTTGCTTCGAGAAAAT
ACAGATGAGGTATTTCCAGATGAACAGCTTAGCGATGGAGAAAAT
GGCATCCCTGTTGGTGTGTCACCAGATAAATTGCCTGGATCTCTG
GGACACCCCGTCCCCAGGAGAAGGATGTTTGGGAAGAGATGGAT
GCCAACAAAAACAAGATAAAGCTTGGAATTTGTAAGGCTGCTACT
GAAGAGGAGAACAGCCATGGCCAGGCAAATGGTCTTCTCAATGCT
CCAAGCCTTGGGTCACCAATTCGTGTCCGCTCAGATTACTCAGCC
AGACAGAGATATTCCACTGGTGCAGAAAGTTACGTTCCATTCACAG
CTTtgagtagcggccgc