Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active Tau Tubulin Kinase 2 [1 – 450]

Enzyme description:- TTBK2 [1 – 450]

Clone number:- DU 19034

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 78, 356.12 daltons
Average Mass 78, 405.95 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 7.02

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 [KKKVSRSGLYRSPSMPENLNRPR] Final concentration: 300 μM

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Clone Data Sheet

TTBK2 [1 – 450]

Protein	TTBK2 [1 - 450]
Clone number	DU 19034
Species	Human
Accession number	BC071556
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGSPEIPGSTRAAA**MSGGGEQPDILSVGILVKERWKVLRKIG** **GGGFGEIYDALDMLTRENVALKVESAQQPKQVLKMEVAVLKKLQG** **KDHVCRFIGCGRNDRFNYVVMQLQGRNLADLRRSQSRGTFTISTT** **LRLGRQILESIESIHSVGFLHRDIKPSNFAMGRFPSTCRKCYMLD** **FGLARQFTNSCGDVRPPRAVAGFRGTVRYASINAHRNREMGRHDD** **LWSLFYMLVEFVVGQLPWRKIKDKEQVGSIKERYDHRLMLKHLPP** **EFSIFLDHISSLDYFTKPDYQLLTSVFDNSIKTFGVIESDPFDWE** **KTGNDGSLTTTTTSTTPQLHTRLTPAAIGIANATPIPGDLLRENT** **DEVFPDEQLSDGENGIPVGVSPDKLPGSLGHPRPQEKDVWEEMDA** **NKNKIKLGICKAATEEENSHGQANGLLNAPSLGSPIRVRSEITQP** **DRDIPLVRKLRSIHSFE**
Native sequence	Amino acids M1 – E450 of human TTBK2. [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	*Not*I sites of pGEX-6P-2

Nucleotide sequence of insert

gcggccgcgATGAGTGGGGGAGGAGAGCAGCCGGATATCCTGAGT
GTTGGAATCCTAGTGAAAGAAAGATGGAAAGTGTTGAGAAAGATT
GGGGGTGGGGGCTTTGGAGAAATTTACGATGCCTTGGACATGCTC
ACCAGGGAAAATGTTGCACTGAAGGTGGAATCAGCTCAACAACCA
AAACAAGTTCTGAAAATGGAAGTTGCTGTTTTGAAAAAGCTGCAA
GGGAAAGACCATGTTTGTAGATTTATTGGCTGTGGGAGGAATGAT
CGATTCAACTATGTGGTCATGCAGTTGCAGGGTCGGAATCTGGCA
GATCTTCGCCGTAGCCAGTCCCGAGGCACATTCACCATTAGTACC
ACTCTCCGGCTGGGTAGACAGATTTTGGAGTCTATTGAAAGCATT
CATTCTGTGGGATTCTTGCATCGAGACATCAAACCGTCGAACTTC
GCTATGGGTCGCTTTCCTAGTACATGTAGGAAATGTTACATGCTT
GATTTTGGCTTGGCTCGACAATTTACCAATTCCTGTGGTGACGTC
AGACCACCTCGAGCTGTGGCAGGTTTTCGAGGGACAGTTCGTTAT
GCATCAATCAACGCACATCGGAACAGGGAAATGGGAAGACATGAT
GACCTTTGGTCCTTATTCTACATGTTGGTGGAGTTTGTGGTTGGT
CAGCTGCCCTGGAGAAAAATAAAGGACAAGGAGCAAGTAGGCTCT
ATTAAGGAGAGATATGACCACAGGCTCATGTTGAAACATCTCCCT
CCAGAATTCAGCATCTTTCTAGACCATATCTCTTCTTTGGATTAT
TTTACAAAACCAGACTACCAGCTTCTTACATCCGTGTTTGACAAT
AGCATCAAGACTTTTGGAGTAATTGAGAGTGACCCTTTTGACTGG
GAGAAGACTGGAAATGATGGCTCCCTAACAACCACCACTACTTCT
ACCACCCCTCAGTTGCACACTCGCTTGACCCCTGCTGCAATTGGA
ATTGCCAATGCTACTCCCATCCCTGGAGACTTGCTTCGAGAAAAT
ACAGATGAGGTATTTCCAGATGAACAGCTTAGCGATGGAGAAAAT
GGCATCCCTGTTGGTGTGTCACCAGATAAATTGCCTGGATCTCTG
GGACACCCCCGTCCCCAGGAGAAGGATGTTTGGGAAGAGATGGAT
GCCAACAAAAACAAGATAAAGCTTGGAATTTGTAAGGCTGCTACT
GAAGAGGAGAACAGCCATGGCCAGGCAAATGGTCTTCTCAATGCT
CCAAGCCTTGGGTCACCAATTCGTGTCCGCTCAGAGATTACTCAG
CCAGACAGAGATATTCCACTGGTGCGAAAGTTACGTTCCATTCAC
AGCTTtgagtaggcggccgc