Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of MYPT3 [1 – 528]

Enzyme description:- MYPT3 [1 – 528]

Clone number:- DU 17842

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 84, 581.92 daltons
Average Mass 84, 635.55 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.70

Purity:- 75 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –70 °C

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Clone Data Sheet

MYPT3 [1 – 528]

Protein	MYPT3 [1 – 528]
Clone number	DU 17842
Species	Human
Accession number	NM_032902
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGS**MAEHLELLAEMPMVGRMSTQERLKHAQKRRAQQVKMWAQ** **AEKEAQGKKGPGERPRKEAASQGLLKQVLFPPSVVLLEAAARNDL** **EEVRQFLGSGVSPDLANEDGLTALHQCCIDDFREMVQQLLEAGAN** **INACDSECWTPLHAAATCGHLHLVELLIASGANLLAVNTDGNMPY** **DLCDDEQTLDCLETAMADRGITQDSIEAARAVPELRMLDDIRSRL** **QAGADLHAPLDHGATLLHVAAANGFSEAAALLLEHRASLSAKDQD** **GWEPLHAAAYWGQVPLVELLVAHGADLNAKSLMDETPLDVCGDEE** **VRAKLLELKHKHDALLRAQSRQRSLLRRRTSSAGSRGKVVRRVSL** **TQRTDLYRKQHAQEAIVWQQPPPTSPEPPEDNDDRQTGAELRPPP** **PEEDNPEVVRPHNGRVGGSPVRHLYSKRLDRSVSYQLSPLDSTTP** **HTLVHDKAHHTLADLKRQRAAAKLQRPPPEGPESPETAEPGLPGD** **TVTPQPDCGFRAGGDPPLLKLTAPAVEAPVERRPCCLLM**
Native sequence	Amino acids M1 – M528 (end) of human. 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 (LEVLFQGP) residues 220 - 227
Cloning sites	*Bam*H1 and *Not*1 sites of pGex6P-1

Nucleotide insert

ggatccaTGGCCGAGCACCTGGAGCTGCTGGCAGAGATGCCCATG
GTGGGCAGGATGAGCACACAGGAGCGGCTGAAGCATGCCCAGAAG
CGGCGCGCCCAGCAGGTGAAGATGTGGGCCCAGGCTGAGAAGGAG
GCCCAGGGCAAGAAGGGTCCTGGGGAGCGTCCCCGGAAGGAGGCA
GCCAGCCAAGGGCTCCTGAAGCAGGTCCTCTTCCCTCCCAGTGTT
GTCCTTCTGGAGGCCGCTGCCCGAAATGACCTGGAAGAAGTCCGC
CAGTTCCTTGGGAGTGGGGTCAGCCCTGACTTGGCCAACGAGGAC
GGCCTGACGGCCCTGCACCAGTGCTGCATTGATGATTTCCGAGAG
ATGGTGCAGCAGCTCCTGGAGGCTGGGGCCAACATCAATGCCTGT
GACAGTGAGTGCTGGACGCCTCTGCATGCTGCGGCCACCTGCGGC
CACCTGCACCTGGTGGAGCTGCTCATCGCCAGTGGCGCCAATCTC
CTGGCGGTCAACACCGACGGGAACATGCCCTATGACCTGTGTGAT
GATGAGCAGACGCTGGACTGCCTGGAGACTGCCATGGCCGACCGT
GGCATCACCCAGGACAGCATCGAGGCCGCCCGGGCCGTGCCAGAA
CTGCGCATGCTGGACGACATCCGGAGCCGGCTGCAGGCCGGGGCA
GACCTCCATGCCCCCCTGGACCACGGGGCCACGCTGCTGCACGTC
GCAGCCGCCAACGGGTTCAGCGAGGCGGCTGCCCTGCTGCTGGAA
CACCGAGCCAGCCTGAGCGCTAAGGACCAAGACGGCTGGGAGCCG
CTGCACGCCGCGGCCTACTGGGGCCAGGTGCCCCTGGTGGAGCTG
CTCGTGGCGCACGGGGCCGACCTGAACGCAAAGTCCCTGATGGAC
GAGACGCCCCTTGATGTGTGCGGGGACGAGGAGGTGCGGGCCAAG
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CCAGTGCGGCATCTATACTCCAAGCGACTAGACCGGAGTGTCTCC
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CACGACAAGGCCCACCACACCCTGGCTGACCTGAAGCGCCAGCGA
GCTGCTGCCAAGCTGCAGCGACCCCCACCTGAGGGGCCCGAGAGC
CCTGAGACAGCTGAGCCTGGCCTGCCTGGTGACACGGTGACCCCC
CAGCCTGACTGTGGCTTCAGGGCAGGCGGGGACCCACCCCTGCTC
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TGCTGCCTGCTCATGtgatgctgaaaaactcgagccatccggaag
atctggcggccgc