

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of MYPT1 [1 – 1030]

Enzyme description:- MYPT1 [1 – 1030]

Clone number:- DU 44600

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 143,068.49 daltons

Average Mass 143,156.05 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.38

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

MYPT1 [1 – 1030]

Protein MYPT1 [1 – 1030]

Clone number DU 44600

Species Human

Accession number NM_001143885.1

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK
FELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERA
EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED
RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL
FQGPLGSPEIPGSTRAAAMKMAKQKRNEQLKRWIGSETDLEPPV
VKRQKTKVKFDDGAVFLAACSSGDTDEVLLKLLHRGADINYANVDG
LTALHQACIDNNDVMVKFLVENGANINQPDNEGWIPLHAAASCGY
LDIAEFLIGQGAHVGAHVNSEGDTPLDIAEEEAMEELLQNEVNROG
VDIEAARKEEERIMLRDARQWLNSGHINDVRHAKSGGTALHVA
AAKGYTEVLKLLIQAGYDVNIKDYGWTPHAAAHWGKEEACRILVD
NLCDMEMVNKVGQTAFDVADEDILGYLEELQKKQNLHSEKRD
KKSPLIESTANMDNNQSOKTFKNKETLIEPEKNASRIESLEQEK
VDEEEGKKDESSCSSEDEEDDSESEAETDKTKPLASVTNANT
SSTQAAPVAVTTPTVSSGQATPTSPIKKFPTTATKISPKEEER
KDESPATWRLGLRKTGSYGALAEITASKEGQKEKDTAGVTRS
ASSPRLSSSLDNKEKEKDSKGRRLAYVAPTIPRRLASTSDIEE
KENRDSSSLRTSSSYTRRKWEDDLKKNSSVNEGSTYHKSCSF
GRRQDDLISSSVSTTSTPTVTSAGLQKSLLSSTSTTTKIT
TGSSSAGTQSSTSNRLWAEDSTEKEKDSVPTAVTIPVAPT
VNVAAASTTTLTITTTAGTVSSTTEVRERRRSYLT
PVRDEESESQRKARSRQARQSRRSTQGVTLTD
LQEAETIGRSRSTRTREQENEEKEKEKEKQDKEKQEEK
KETSRDEYKQKYSRTYDETYQRYRPVSTSSSTTPSSSL
STMSSSLYASSQLNRPNLSLVGITSAYSRGITKENERE
GEGKREEKEGEDKSQPKSIRERRRPREKRRSTGVS
FWTQDSDENEQEQQSDTEEGSNKKETQTD
SISRYETSSTSAGDRYDLSLLGRSGSYSLEERKPY
SSRLEKDDSTDFKKLYEQILAENEKLKAQLHDTN
MELTDLKLQLEKATQRQERFADRSLLEMEKR
RERALERRISEMEEELKMLPDLKADNQRLK
DENGALIRVISKLSK

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<u>Native sequence</u>	Amino acids M1 – K1030 (end) of human. Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 220 - 227
<u>Cloning sites</u>	<i>Not1</i> sites of pGex6P-2

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Nucleotide insert

gcggccgcgATGAAGATGGCGGACGCGAAGCAGAAGCGGAACGAG
CAGCTGAAACGCTGGATCGGCTCCGAGACGGACCTCGAGCCTCCG
GTGGTGAAGCGCCAGAAGACCAAGGTGAAGTTCGACGATGGCGCC
GTCTTCCTGGCTGCTTGCTCCAGCGGCGACACGGACGAGGTCCTC
AAGCTGCTGCACCGCGGCGCCGACATCAATTACGCCAATGTGGAC
GGACTCACTGCCCTGCACCAGGCTTGCAATTGATGACAATGTTGAT
ATGGTGAAGTTCCTGGTAGAAAATGGAGCAAATATTAATCAACCT
GATAATGAAGGCTGGATACCCCTACATGCAGCAGCTTCCTGTGGA
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GGGGCTGTCAACAGTGAAGGAGATACACCTTTAGATATTGCGGAG
GAGGAGGCAATGGAAGAGCTACTTCAAAATGAAGTTAATCGGCAA
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CTTAGAGATGCCAGGCAGTGGCTAAATAGTGGTCATATAAATGAT
GTCCGGCATGCAAAATCTGGAGGTACAGCACTTCACGTTGCAGCT
GCTAAAGGCTATACGGAAGTTTTTAAACTTTTAATACAGGCAGGC
TATGATGTTAATATTAAGACTATGATGGCTGGACACCTCTTCAT
GCTGCAGCTCATTGGGGTAAAGAAGAAGCATGTCGAATTTTAGTG
GACAATCTGTGTGATATGGAGATGGTCAACAAAGTGGGCCAAACA
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ACGGCAGTGACCATTCCTGTTGCTCCAACGTGTTGTAAATGCTGCA
GCTTCTACCACAACCCTGACTACAACCTACTGCTGGCACTGTCTCC
TCCACAACAGAGGTCAGGGAGAGACGCAGATCATACCTCACTCCT
GTTAGGGATGAAGAGTCTGAATCCCAAAGAAAAGCAAGATCTAGA
CAAGCAAGACAATCTAGAAGATCAACACAGGGAGTGACATTAAC

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GATCTTCAAGAAGCTGAGAAAACAATAGGAAGAAGTCGTTCTACC
CGAACCAGAGAACAAGAAAATGAAGAAAAAGAAAAAGAGGAAAAA
GAGAAACAAGATAAAGAGAAACAAGAAGAAAAGAAGGAGTCAGAA
ACATCTAGAGAAGATGAATATAAACAAAAGTACTCCAGAACGTAT
GATGAGACTTACCAGCGTTATAGGCCAGTATCAACTTCAAGTTCA
ACCACTCCATCCTCTTCACTTTCTACTATGAGCAGTTCACTGTAT
GCTTCAAGTCAACTAAACAGGCCAAATAGTCTTGTAGGCATAACT
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GAACAACAATCAGACACAGAAGAGGGATCCAATAAGAAAAGAACT
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GACTCAACTGACTTTAAAAAGCTTTATGAACAAATTCTAGCTGAA
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AGATTTGCTGATAGATCACTGTTGGAAATGGAAAAAAGGGAACGA
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AATGGGGCCTTGATCAGAGTTATAAGCAAACCTTTCCAAAtaagcg
gccgc