

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of MYPT1 [375 - 974]

<u>Protein description:-</u>	MYPT1 [375 - 974]
<u>Clone number:-</u>	DU 1887
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose, followed by Q-Sepharose
<u>Expression level:-</u>	0.5 mg/L
<u>Calculated molecular mass:-</u>	95, 458 daltons
<u>Purity:-</u>	75 %
<u>Enzyme storage buffer:-</u>	50 mM Tris/HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-20 °C
<u>Assay:-</u>	Substrate for ROK alpha

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CLONE DATA SHEET

MYPT1 [375 - 974]

<u>Protein</u>	MYPT1 [375 - 974]
<u>Clone number</u>	DU 1887
<u>Species</u>	Rat
<u>Accession number</u>	NM_053890
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAIEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAIPIQIDKYLKSSKYIAWPLQG WQATFGGGDHPKSDLVPRGSPRYISGGGGGILDSMASVTN AHTASTQAAPAAVTTPTLSSNQGTPVVKKFPSTTKISP KEEERKDESPASWRLGLRKTGSYGALAEITASKEAQEKDT AGVIRSASSPRLSSSLDNKEKEKDNKGTRLAYVAPTIPRRL GSTSDIEEKENRESSNLRTSSSYTRRKWEDDLKKNSSINEG STYHRSTSNRLWAEDSTEKEKDSAPTAATILVAPTIVSAAA SSTTALTTTGTAGTLSSTSEVRERRRSYLTTPVRDEESESQRK ARSRQARQSRRSTQGVTLDLQEAETIGRSRSTRTREQEN EKDKEEKEKQDKEKQEEKKESEVSREDEYKQKYSRTYDET YARYRPVSTSSSSSTPSSSSLSTLGSSLYASSQLNRPNSLVG ITSAYSRGLTKDNEREGEKKEEKEGEDKSQPKSIRERRRP REKRRSTGVSFWTQSDENEQERQSDTEDGSSKRDTQTDV SRYDSSSTSSSDRYDSLGRSASYSYLEERKPYGSRLEKDD STDFKKLYEQILAENEKLAQLHDTNMELTDLKLQLEKATQ RQERFADRSLLEMEKRERRALERRISEMEEELKMLPDLKAD NQRLKDENGALIRVISKLSK</p>
<u>Native sequence</u>	Amino acids M375 – K974 (end) of rat MYPT1. Residue M241 of the fusion protein is equivalent to M375 of the native protein. The GST tag is located at residues 1 – 220
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 221 - 226

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Cloning sites

*Bam*HI and *Sal*I sites of pGEX 4T

Nucleotide sequence of insert

GGATCCCCGCGGTATATTTCCGGTGGTGGTGGTGGAAATTCTAGACTCC
ATGGCTTCTGTAACATAATGCTCACACTGCCAGCACTCAGGCAGCTCCT
GCCGCTGTGACAACACCTACTCTGTCTTCCAACCAGGGGACCCCTACA
TCACCTGTTAAAAAGTTTCCCTACATCAACTACAAAAATTTCTCCAAA
GAAGAAGAAAGAAAAGATGAATCTCCTGCATCCTGGAGGTTAGGACTT
AGAAAGACTGGCAGTTATGGTGCCCTGGCTGAGATCACTGCATCTAAA
GAAGCTCAGAAGGAGAAAGACACTGCAGGCGTGATACGTTTCAGCTTCG
AGTCCCAGACTCTCGTCCTCTTTGGATAATAAAGAAAAGGAGAAAGAC
AATAAAGGAACAAGACTTGCATATGTGCCCCCTACAATCCCAAGGCGA
CTAGGCAGTACGTCTGACATTGAAGAGAAGGAAAACAGAGAGTCTTCA
AATTTGCGAACAAAGTAGTTCTTACACAAGAAGAAAATGGGAAGATGAT
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AGTGCTCCTACCGCAGCGACCATTTCTTGTGTCTCCAAGTGTGTAAGT
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CTTTCCTCCACATCAGAGGTCAGGGAGAGACGCAGGTCATACCTCACT
CCTGTTAGGGATGAAGAGTCTGAATCCCAAAGGAAAGCAAGATCTAGA
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