

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of MYPT1 [714 - 1004]

<u>Protein description:-</u>	MYPT1 [714 - 1004]
<u>Clone number:-</u>	DU 652
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal Maltose Binding Protein (MBP) and HA
<u>Purification method:-</u>	Amylose agarose
<u>Expression level:-</u>	1 mg/L
<u>Calculated molecular mass:-</u>	81, 406 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 [714 - 1004]

<u>Protein</u>	MYPT1 [714 - 1004]
<u>Clone number</u>	DU 652
<u>Species</u>	Chicken
<u>Accession number</u>	D37985
<u>Tags</u>	N-terminal MBP and HA
<u>Bacterially expressed protein</u>	MKIKTGARILALSALTMMFSASALAKIEEGKLVIWINGDKGYNGLAE VGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGY AQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYN KDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGG YAFKYENGKYDIKDVGVNAGAKAGLTFLVDLIKHKHMNADTDYSIAE AAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGV SAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYYY LAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTV EALKDAQTNSSSNNNNNNNNNNLGIEGRISEFMAYPYDVPDCASLGGH MARIRSTRTREQENEEKEKEKEKEQDKEKQEEKKESETKDDDYRQRY RTVEEPYHRYRPTSTSTSTSSSLSTSTSSSSQLNRPNSLIGIT SAYSRSRGTKESEREGGKKEEKEEDKSQPKSIRERRRPREKRRSTGVS FWTQSDENEQEHSQDSEEGTNKKETQSDSLSRDYTGSLSVSSGDRYD SAQGRSGSQSYLEDKPYCSRLEKEDSTDFKKLYEQILAENEKLKAQL HDTNMELTDLKLQLEKTTQRQERFADRSLLEMEKRVSGKSQYLLGGKK SSRKKDI
<u>Native sequence</u>	Amino acids R714 – I1004 (end) of chicken MYPT1. Residue R714 of the fusion protein is equivalent to R437 of the native protein. The MBP tag is located at residues 1 – 408 and the HA tag is located at residues 419 – 427. The following sequence is present after the HA tag, SLGGHMARI, residues 428 - 436.
<u>Protease cleavage</u>	Factor Xa (<u>IEGR</u>) at residues 409 - 412
<u>Cloning sites</u>	<i>Eco</i> RI sites of pMAL p2X

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Nucleotide
sequence of insert

GAATTCATGGCTTACCCATACGATGTTCCAGATTGCGCTAGCTTGGGT
GGTCATATGGCTAGAATTCGTTCCACACGAACCAGAGAACAAGAAAAT
GAAGAAAAAGAAAAGGAAGAGAAAAGAAAAGCAAGACAAAGAAAAACAA
GAAGAAAAGAAAGAATCTGAAACTAAAGATGATGATTATAGACAAAGA
TATTCCCGAACTGTTGAAGAGCCATATCATCGCTATAGACCAACTTCA
ACTTCAACTTCTACATCATCCACCTCTTCGCTCTCCACCTCCACTAGC
TCTCTTTCAAGTTCAAGTCAGCTAAACAGACCAAACAGCCTTATAGGT
ATAACTTCTGCCTATTCTCGGAGTGGAACAAAGGAAAGTGAGAGAGAA
GGGGGCAAAAAGAGGAAGAGAAGGAGGAAGATAAGTCACAGCCCAAA
TCTATACGGGAAAGGCGGCGACCAAGAGAAAAACGGCGATCTACAGGA
GTTTCATTTTGGACTCAAGATAGTGATGAAAATGAACAGGAGCACCAG
TCTGATTCAGAAGAAGGAACAAATAAGAAAGAAACTCAGAGTGATAGC
CTTTCAAGATATGACACGGGATCACTCAGTGTGTCTTCGGGTGATCGT
TACGATTCAGCACAGGGGCGATCTGGATCACAGAGTTACCTTGAAGAT
CGAAAACCTTACTGCAGTAGGCTAGAAAAAGAAGATAGCACTGATTTT
AAGAAGCTTTATGAACAGATCCTAGCTGAAAACGAAAAGCTGAAAGCA
CAGTTACATGATACCAACATGGAACCTTACAGATCTTAAACTACAGCTG
GAGAAGACCACTCAGAGACAAGAAAGATTTGCTGACAGATCGCTCCTG
GAAATGGAGAAAAGGGTGTCCGGCAAGAGTCAGTATCTACTGGGCGGA
AAGAAGAGCTCTAGAAAGAAGGATATCtga