

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PP5 [1 – 499]

<u>Enzyme description:-</u>	PP5 [1 – 499]
<u>Clone number:-</u>	DU 1727
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST and C-terminal EE
<u>Purification method:-</u>	GST Sepharose
<u>Expression level:-</u>	5 mg/L
<u>Calculated molecular mass:-</u>	84, 281 daltons
<u>Purity:-</u>	90 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
	50 mM Tris-HCl pH 7.5, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 50 % glycerol, 1 mM benzamidine and 0.1 mM PMSF
<u>Storage temperature:-</u>	-20 °C
<u>Assay:-</u>	Standard phosphatase assay
<u>Assay buffer:-</u>	
	50 mM Tris-HCl pH 7.5, 3 mM MnCl ₂ , 0.03 % Brij 35, 0.1 mM EGTA, 200 μM Arachidonic acid, 0.1 % 2-mercaptoethanol
<u>Substrate:-</u>	
	[³² P] casein: phosphorylated by PKA Final concentration: 6 μM
<u>Specific activity range:-</u>	50 – 100 mU/mg

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Clone Data Sheet

PP5 [1 – 499]

<u>Protein</u>	PP5 [1 – 499]
<u>Clone number</u>	DU 1727
<u>Species</u>	Human
<u>Accession number</u>	NM_006247
<u>Tags</u>	N-terminal GST and C-terminal EE (EFMPME)
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLRYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFED RLCHKTYLNGDHVTHPDFMFLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRG SPNSTMAMAEGERTECAEPPRDEPPADGALKRAEELKTQANDYFK AKDYENAIFYSQAIELNPSNAIYYGNRSLAYLRTECYGYALGDA TRAIELDKKYIKGYRRAASNMALGKFRAALRDYETVVKVPHDK DAKMKYQECNKIVKQKAFERAIAGDEHKRSVVDSLDIESMTIEDE YSGPKLEDGKVTISFMKELMQWYKDQKKLHRKCAYQILVQVKEVL SKLSTLVETTLKETEKITVCGDTHGQFYDLLNIFELNGLPSETNP YIFNGDFVDRGSFSVEVILTLFGFKLLYPDHFHLLRGNHETDNMN QIYGFEGEVKAKYTAQMYELFSEVFEWLPLAQCINGKVLIMHGGL FSEDGVTLD DIRKIERNRQPPDSGPMCDLLWSDPQPQNGRSISKR GVSCQFGPDVTKAFLEENLDYIIRSHEVKAEGYEVAHGGRCVTV FSAPNYCDQMGNKASYIHLQGSDLRPQFHQFTAVPHPNVKPMAYA NTLLQLGMMEFMPME</p>
<u>Native sequence</u>	<p>Amino acids M1 – M499 (end) of human PP5. Residue M231 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220 and the EE tag is located at residues 730 – 735.</p>
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 221 - 226
<u>Cloning sites</u>	<i>Bam</i> H1 sites of pGEX 4T

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Nucleotide
Sequence of insert

GGATCCCCGAATTCCACCATGGCGATGGCGGAGGGCGAGAGGACT
GAGTGTGCTGAGCCCCCGGGACGAACCCCGGCTGATGGAGCT
CTGAAGCGGGCAGAGGAGCTCAAGACTCAGGCCAATGACTACTTC
AAAGCCAAGGACTACGAGAACGCCATCAAGTTCTACAGCCAGGCC
ATCGAGCTGAACCCAGCAATGCCATCTACTATGGCAACCCGAGC
CTGGCCTACCTGCGCACTGAGTGCTATGGCTACGCGCTGGGAGAC
GCCACGCGGGCCATTGAGCTGGACAAGAAGTACATCAAGGGTTAT
TACCGCCGGGCTGCCAGCAACATGGCACTGGGCAAGTTCCGGGCC
GCGCTGCGAGACTACGAGACGGTGGTCAAGGTGAAGCCCCATGAC
AAGGATGCCAAAATGAAATACCAGGAGTGCAACAAGATCGTGAAG
CAGAAGGCCTTTGAGCGGGCCATCGCGGGCGACGAGCACAAGCGC
TCCGTGGTGGACTCGCTGGACATCGAGAGCATGACCATTGAGGAT
GAGTACAGCGGACCCAAGCTTGAAGACGGCAAAGTGACAATCAGT
TTCATGAAGGAGCTCATGCAGTGGTACAAGGACCAGAAGAACTG
CACCGGAAATGTGCCTACCAGATTCTGGTACAGGTCAAAGAGGTC
CTCTCCAAGCTGAGCACGCTCGTGAAACCACACTCAAAGAGACA
GAGAAGATTACAGTATGTGGGGACACCCATGGCCAGTTCTATGAC
CTCTCAACATATTCGAGCTCAACGGTTTACCCTCGGAGACCAAC
CCCTATATATTTAATGGTGACTTTGTGGACCGAGGCTCCTTCTCT
GTAGAAGTGATCCTCACCTTTTCGGCTTCAAGCTCCTGTACCCA
GATCACTTTCACCTCCTTCGAGGCAACCACGAGACAGACAACATG
AACCAGATCTACGGTTTCGAGGGTGAGGTGAAGGCCAAGTACACA
GCCAGATGTACGAGCTCTTTAGCGAGGTGTTTCGAGTGGCTCCCG
TTGGCCCAGTGCATCAACGGCAAAGTGCTGATCATGCACGGAGGC
CTGTTCAGTGAAGACGGTGTACCCTGGATGACATCCGAAAATT
GAGCGGAATCGACAACCCCCAGATTCAGGGCCCATGTGTGACCTG
CTCTGGTCAGATCCACAGCCACAGAACGGGCGCTCGATCAGCAAG
CGGGGCGTGAGCTGTCAGTTTGGGCCTGACGTCACCAAGGCCTTC
TTGGAAGAGAACAACCTGGACTATATCATCCGCAGCCACGAAGTC
AAGCCGAGGGCTACGAGGTGGCTCACGGAGGCCGCTGTGTACC
GTCTTCTCTGCCCCAACTACTGCGACCAGATGGGGAACAAAGCC
TCCTACATCCACCTCCAGGGCTCTGACCTACGGCCTCAGTTCCAC
CAGTTACAGCAGTGCTCATCCCAACGTCAAGCCCATGGCCTAT
GCCAACACGCTGCTGCAGCTAGGAATGATGGAATTTATGCCGATG
GAAtgaattc