

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PP1 alpha [1 – 330]

Enzyme description:- PP1 alpha [1 - 330]

Clone number:- DU 46517

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 64, 294.44 daltons

Average Mass 64, 336.24 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.84

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 2 mM MnCl₂,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

PP1 alpha [1 - 330]

<u>Protein</u>	PP1 alpha [1 - 330]
<u>Clone number</u>	DU 46517
<u>Species</u>	Human
<u>Accession number</u>	NM_002708.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMDSSEKLNLDSEIIG RLLEVQGSRPGKNVQLTENEIRGLCLKSREIFLSQPILLELEAPLKICG DIHQYYDLLRLFYGGFPPESNYLFLGDYVDRGQSLETICLLLAYKI KYPENFFLLRGNHECASINRIYGFYDECKRRYNIKLWKTFTDCFNCLPI AAIVDEKIFCCHGGLSPDLQSMEQIRRMPTDVPDQGLLCDLLWSDPD KDVQGWGENDRGVSFTFGAEVVAKFLHKHDLDLICRAHQVVEDGYEFFA KRQLVTLFSAPNYCGEFDNAGAMMSVDETLMCSEFQILKPADKNKGKYGO FSGLNPGGRPITPPRNSAKAKK
<u>Native sequence</u>	Amino acids M1 – K330 (end) of human PP1 alpha. Residue M232 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 (LEVLFGQP) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide

Sequence of insert

ggatccATGTCCGACAGCGAGAAGCTCAACCTGGACTCTATCATCGGGC
GCCTGCTGGAAGTGCAGGGCTCGCGGCCCGGAAAGAATGTGCAGCTGAC
GGAGAACGAGATCCGTGGTCTGTGCCTCAAATCCCGGGAGATCTTCCTG
AGCCAGCCCATTCTGCTGGAGCTGGAGGCGCCCCTCAAGATCTGCGGTG
ACATCCACGGCCAATACTACGACCTGCTGCGGCTGTTTCGAGTACGGCGG
CTTCCCCCAGAGAGCAACTACCTGTTCTGGGTGACTACGTGGACCGC
GGCAAGCAGTCCCTGGAGACCATCTGCCTGCTGCTGGCCTACAAGATCA
AGTACCCCGAGAACTTCTTCCTGCTGCGCGGGAACACGAGTGCGCCAG
CATCAACCGCATCTACGGCTTCTACGACGAGTGCAAGAGACGCTACAAC
ATCAAGCTGTGGAAGACGTTCAACGACTGCTTCAACTGCCTGCCCATCG
CGGCCATTGTGGACGAGAAGATATTCTGCTGCCATGGCGGCCTCTCCCC
CGACCTGCAGTCCATGGAGCAGATCCGGCGCATCATGCGGCCCCACGGAC
GTGCCCCGACCAGGGCCTGCTGTGTGACCTGCTGTGGTCTGACCCCGACA
AGGACGTGCAGGGCTGGGGCGAGAACGACCGCGGCGTCTCCTTCACCTT
CGGCGCGGAGGTGGTGGCCAAGTTCCTGCACAAGCATGACCTGGACCTC
ATCTGCCGGGCGCACCAGGTGGTGGAGGACGGCTATGAGTTCTTTGCCA
AGCGGCAGCTGGTGACACTTTTCTCAGCCCCCACTACTGTGGCGAGTT
CGACAACGCCGGAGCCATGATGAGCGTGGACGAGACCCTCATGTGCTCC
TTCCAGATCCTTAAGCCGGCCGACAAGAACAAGGGCAAGTACGGGCAGT
TCAGTGGCCTGAACCCTGGAGGCCGACCCATCACCCACCCCGCAACTC
TGCCAAAGCCAAGAAAtaggcggccgc