

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PP1 gamma [2 – 323]

Enzyme description:- PP1 gamma [2 - 323]

Clone number:- DU 807

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 63, 635.32 daltons

Average Mass 63, 676.76 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.93

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 gamma [2 - 323]

<u>Protein</u>	PP1 gamma [2 - 323]
<u>Clone number</u>	DU 807
<u>Species</u>	Human
<u>Accession number</u>	NM_002710.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVFGQPLGSADLDKLNIDSI IQR LLEVRGSKPGKNVQLQENEIRGLCLKSREIFLSQPILLELEAPLKICGD IHGQYYDLLRLFYGGFPPESNYFLGDYVDRGKQSLETICLLLAYKIK YPENFFLLRGNHECASINRIYGFYDECKRRYNIKLWKTFTDCFNCLPIA AIVDEKIFCCHGGLSPDLQSMEQIRRIMRPTDVPDQGLLCDLLWSDPDK DVLGWGENDRGVSFTFGAEVVAKFLHKHDLDLICRAHQVVEDGYEFFAK RQLVTLFSAPNYCGEFDNAGAMMSVDETLMCSFQILKPAEKKKPNATRP VTPPRGMITKQAKK</p>
<u>Native sequence</u>	<p>Amino acids A2 – K323 (end) of human PP1 gamma. Residue M232 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLVFGQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pGEX6P-1

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Nucleotide
Sequence of insert

ggatccgCGGATTTAGATAAACTCAACATCGACAGCATTATCCAACGGC
TGCTGGAAGTGAGAGGGTCCAAGCCTGGTAAGAATGTCCAGCTTCAGGA
GAATGAAATCAGAGGACTGTGCTTAAAGTCTCGTGAAATCTTTCTCAGT
CAGCCTATCCTACTAGAACTTGAAGCACCACTCAAAATATGTGGTGACA
TCCATGGACAATACTATGATTTGCTGCGACTTTTTGAGTACGGTGGTTT
CCCACCAGAAAGCAACTACCTGTTTCTTGGGGACTATGTGGACAGGGGA
AAGCAGTCATTGGAGACGATCTGCCTCTTACTGGCCTACAAAATAAAAT
ATCCTGAGAATTTTTTTCTTCTCAGAGGGAACCATGAATGTGCCAGCAT
CAACAGAAATTTATGGATTTTATGATGAATGTAAAAGAAGATACAACATT
AAACTATGGAAAACTTTCACAGACTGTTTTAACTGTTTACCGATAGCAG
CCATCGTGGATGAGAAGATATTCTGCTGTCATGGAGGTTTATCACCAGA
TCTTCAATCTATGGAGCAGATTCGGCGAATTATGCGACCAACTGATGTA
CCAGATCAAGGTCTTCTTTGTGATCTTTTGTGGTCTGACCCCGATAAAG
ATGTCTTAGGCTGGGGTGAAAATGACAGAGGAGTGTCTTCACATTTGG
TGCAGAAGTGGTTGCAAAATTTCTCCATAAGCATGATTTGGATCTTATA
TGTAGAGCCCATCAGGTGGTTGAAGATGGATATGAATTTTTTGC AAAGA
GGCAGTTGGTCACTCTGTTTTCTGCGCCAATTATTGCGGAGAGTTTGA
CAATGCAGGTGCCATGATGAGTGTGGATGAAACACTAATGTGTTCTTTT
CAGATTTTAAAGCCTGCAGAGAAAAAGAAGCCAAATGCCACGAGACCTG
TAACGCCTCCAAGGGGTATGATCACAAAGCAAGCAAAGAAAtaggaatt

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