

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PAF1 [1 – 531]

<u>Enzyme description:-</u>	PAF1 [1 - 531]
<u>Clone number:-</u>	DU 17781
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 86, 745.74 daltons
Average Mass 86, 799.88 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.69

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
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

PAF1 [1 - 531]

<u>Protein</u>	PAF1 [1 - 531]
<u>Clone number</u>	DU 17781
<u>Species</u>	Human
<u>Accession number</u>	NM_019088.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWOATFGGGDHPPKSDLEVLFGQGPLGSMAPTIQTQAQREDG HRPNSHRTLPERSGVVCRVKYCNSLPDIPFDPKFITYPFDQNRVQYKA TSLEKQHKHDLLTEPDLGVTIDLINPDITYRIDPNVLLDPADEKLLEEEI QAPTSSKRSQQHAKVVPWMRKTEYISTEFNRYGISNEKPEVKIGVSVKQ QFTEEEIYKDRDSQITAIEKTFEDAQKSISQHYSKPRVTPVEVMPVFPD FKMWINPCAQVIFDSDPAPKDTSGAAALEMMSQAMIRGMMDEEGNQFVA YFLPVEETLKKRKRQEEEMDYAPDDVYDYKIAREYNWNVKNKASKGYE ENYFFIFREGDGVYNELETRVRLSKRRAKAGVQSGTNALLVVKHRDMN EKELEAQEARKAQLENHEPEEEEEEMETEEKEAGGSDEEQEKGSSEK EGSEDEHSGSESEREEGDRDEASDKSGSGEDESEDEARAARDKEEIFG SDADSEDDADSDDEDRGQAQGGSDNDSDSGSNGGGQRSRSHRSASFPF SGSEHSAQEDGSEAAASDSSEADSDSD
<u>Native sequence</u>	Amino acids M1 – D531 (end) of human PAF1. 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

ggatccATGGCGCCCACCATCCAGACCCAGGCCAGCGGGAGGATGGCCACAGGCCCAATTCCCAC
CGGACTCTGCCTGAGAGGTCTGGAGTGGTCTGCCGAGTCAAGTACTGCAATAGCCTCCCTGATATC
CCCTTCGACCCCAAGTTCATCACCTACCCCTTCGACCAGAACAGGTTCGTCCAGTACAAAGCCACT
TCCTTGAGAAACAGCACAAACATGACCTCCTGACTGAGCCAGACCTGGGGGTCACCATCGATCTC
ATCAATCCTGACACCTACCGCATCGACCCCAATGTTCTTCTAGATCCAGCTGATGAGAACTTTTG
GAAGAGGAGATTCAGGCCCCCACCAGCTCCAAGAGATCCCAGCAGCACGCGAAGGTGGTGCCATGG
ATGCGAAAGACAGAGTACATCTCCACTGAGTTCAACCGTTATGGCATCTCCAATGAGAAGCCTGAG
GTCAAGATTGGGGTTTCTGTGAAGCAGCAGTTTACCGAGGAAGAAATATACAAAGACAGGGATAGC
CAGATCACAGCCATTGAGAAGACTTTTGAGGATGCCCAGAAATCAATCTCACAGCATTACAGCAA
CCCCGAGTCACACCGGTGGAGGTCATGCCTGTCTTCCCAGACTTTAAGATGTGGATCAATCCATGT
GCTCAGGTGATCTTTGACTCAGACCCAGCCCCAAGGACACGAGTGGTGCAGCTGCGTTGGAGATG
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CCTGTAGAAGAGACGTTGAAGAAACGAAAGCGGGACCAGGAGGAGGAGATGGACTATGCACCAGAT
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TATGAGGAAAATACTTCTTCATCTTCCGAGAGGGTGACGGGGTTTACTACAATGAGTTGGAAACC
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AACCACGAACCGGAGGAGGAAGAGGAAGAGGAGATGGAGACAGAAGAGAAAGAAGCTGGGGGCTCA
GATGAGGAGCAGGAGAAGGGCAGCAGCAGTGAGAAGGAGGGCAGTGAAGATGAGCACTCGGGCAGC
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GAGGACGATGCCGACTCTGATGATGAGGACAGAGGACAGGCCCAAGGTGGCAGTGACAATGATTCA
GACAGCGGCAGCAATGGGGGTGGCCAGCGGAGCCGGAGCCACAGCCGCAGCGCCAGTCCCTTCCCC
AGTGGCAGCGAGCACTCGGCCAGGAGGATGGCAGTGAAGCTGCAGCTTCTGATTCCAGTGAAGCT
GATAGTGACAGTGACTgagcggccgc

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