

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PSF [1 – 707]

<u>Enzyme description:-</u>	PSF [1 - 707]
<u>Clone number:-</u>	DU 19508
<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 103, 615.62 daltons

Average Mass 103, 681.25 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.86

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

PSF [1 - 707]

<u>Protein</u>	PSF [1 - 707]
<u>Clone number</u>	DU 19508
<u>Species</u>	Human
<u>Accession number</u>	NM_005066.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSPNSRVDMSRDRFRS RGGGGGGFHRRGGGGGRGGLHDFRSPPPGMGLNQRGPMGPGPGQSGPK PPIPPPPHQOOQPPPPQPPPHQPPHPQPHQOOQPPPPQDS SKPVVAQGGPAPGVGSTPPASSAPPATPPTSGAPP GSGPGPTPTPPP AVTSAPPGAPPPTPPSSGVPTTPPQAGGPPPPAAVPGPGPKQGGP GGPKGGKMPGGPKGGGGLSTPGGHPKPPRRGGGEPRGGRQHHPYHQ QHHQPPPGGPGGRSEEKISDSEGFKANLSLLRRPGEKTYTQRCRLFVG NLPADITEDEFKRLFAKYGEPGEVFINKGKGFIFIKLESRALAEIAKAE LDDTPMRGRQLRVRFATHAALSVRNLSPYVSNELLEAFSQFGPIERA VVIVDDRGRSTGKGIVFASKPAARKAFERCSEGVFLTTTPRPVIVEP LEQLDDEDGLPEKLAQKNPMYQKERETPTRFAQHGTFEYEYSQRWKS LD EMEKQOREQVEKNMKDAKDKLESEMEDAYHEHQANLLRQDLMR RQEELR RMEELHNQEMQKRKEMQLRQEEERRRREEMMI RQREMEDQMR RQREES YSRMGYMDPRERDMRMGGGGAMNMGDPYSGGGQKFPPLGGGGIGYEAN PGVPPATMSGSMGSDMRTERFGQGGAGPVGGQGPRGMGPGT PAGYGRG REEYEGPNKKPRF</p>
<u>Native sequence</u>	<p>Amino acids M1 – F707 (end) of human PSF. Residue M238 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVL FQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Sal</i> I and <i>Xho</i> I sites into <i>Sal</i> I of pGEX6P-1

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Nucleotide Sequence of insert

gtcgcacATGTCTCGGGATCGGTTCCGGAGTCGTGGCGGTGGCGGTGGTGGCTTCCACAGGCGTGGA
GGAGGCGGCGGCCGCGGCCCTCCACGACTTCCGTTCTCCGCCGCCCGGCATGGGCCTCAATCAG
AATCGCGGCCCATGGGTCCTGGCCCGGGCCAGAGCGGCCCTAAGCTCCGATCCCGCCACCGCCT
CCACACCAACAGCAGCAACAGCCACCACCGCAGCAGCCACCAGCCGCGCAGCAGCCGCCACCGCATCAG
CCGCCCGGCATCCACAGCCGCATCAGCAGCAGCAGCCGCCGCCACCAGCCGCGCAGGACTCTTCCAAG
CCCGTCGTTGCTCAGGGACCCGGCCCCGCTCCCGGAGTAGGCAGCACACCACCAGCCTCCAGCTCG
GCCCCGCCCGCCACTCCACCAACCTCGGGGGCCCCGCCAGGGTCCGGGCCAGGCCCGACTCCGACC
CCGCCGCTGCAGTCACCTCGGCCCTCCCGGGGCGCCGCCACCACCCCGCCAAGCAGCGGGGTC
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GGGCCTAAGCAGGGCCCAGGTCCGGGTGGTCCCAAAGGCGGCAAAATGCCTGGCGGGCCGAAGCCA
GGTGGCGGCCCGGGCCTAAGTACGCCTGGCGGCCACCCCAAGCCGCCGCGTCGAGGCGGCGGGGAG
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AAAAACCCCGATTTtagctcgag

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