

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of SAP97 [1 – 893]

Protein description:- SAP97 [1 – 893]

Clone number:- DU 391

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:- 125, 636 daltons

Purity:- > 90 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- –20 °C

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CLONE DATA SHEET

SAP97 [1 – 893]

Protein SAP97 [1 – 893]

Clone number DU 391

Species Mouse

Accession no AAN87264

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLVPRGSM**MPVRKQDTQRALHL**
LEEYRSKLNQTEDRQLR**SSI**ERVI**SIFQSNLFQALIDIQEFYEV**TL**LLD**
NPKCVDHSKQCEPVQ**PGNPWESGSLSSAAVTSESLPGLSPPVEKYRY**
QDEEVLPSERISPQVPNEVLGP**ELVHVSEKSLSEI**ENVHG**FVSHSHIS**
PIKANPPPVLVNTDSLETPT**YVNGTDADYEYEEITLERGNSGLGFSIA**
GGTDNPHIGDDSSIFITKIITGAAAQDGRLRVND**CILRVNEADVRDV**
THSKAVEALKEAGSIVRLYVKRRKPASEKIMEIKLIK**GPGLGFSIAG**
GVGNQHIPGDNSI**YVTKIIEGGA**AHKDGK**LQIGDKLLAVNSVCLEEV**T
HEEAVTALKNTSDFVYLKVAKPTSMYINDGYAPPDITNSSSQSVDNHV
SPSSYLGQTPASPARYSPISKAVLG**DDEITREPRKVVLRH**RGSTGLGF**N**
IVGGEDGEGIFISFILAGGPADLSGELRKGDRIISVNSVDLRAAS**HEQ**
AAAALKNAGQAVTIVAQYRPEEYSRFEAKIHDLRE**QMMNSSVSSGSGS**
LRTSQKRSLYVRALFDYDKTKDSGLPSQGLN**FKFGDILHVINASDDEW**
WQARQVTPDGESDEGVIPSKRRVEKKERAR**LKTVKFNSKTRGDKGSF**
NDKRKKNLFSRKFPFYKNKDQSEQETSDADQHVT**SNASDSESSYRGQE**
EYVLSYEPVNQQEVNYTRPVIILGPMKDRVNDDLISE**FPDKFGSCVPH**
TTRPKRDYEVDGRDYHFVTSREQMEKDIQEHKFIEAGQYNNHLYG**T**SV
QSVRAVAEKGKHCILDVSGNAIKRLQIAQLYPISTFIKPKSMEN**IMEM**
NKRLTDEQARKTFERAVR**LEQEFTEHFTAIVQGD**TLEDIYNQVKQ**II**E
EQSGPYIWPAKEKL

Native sequence Amino acids M1 – L893 (end) of mouse SAP97.
Residue M227 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 – 220

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The following amino acid substitutions are present:

S – N, where S23 of the native enzyme is N249 of the fusion protein
N – S, where N39 of the native enzyme is S265 of the fusion protein
S – G, where S79 of the native enzyme is G305 of the fusion protein
T – N, where T80 of the native enzyme is N306 of the fusion protein
T – I, where T81 of the native enzyme is I307 of the fusion protein
I – S, where I84 of the native enzyme is S310 of the fusion protein
A – G, where A85 of the native enzyme is G311 of the fusion protein
P – S, where P88 of the native enzyme is S314 of the fusion protein
T – A, where T90 of the native enzyme is A316 of the fusion protein
T – S, where T96 of the native enzyme is S322 of the fusion protein
S – G, where S100 of the native enzyme is G326 of the fusion protein
P – S, where P118 of the native enzyme is S344 of the fusion protein
H – R, where H120 of the native enzyme is R346 of the fusion protein
T – P, where T126 of the native enzyme is P351 of the fusion protein
N – S, where N141 of the native enzyme is S367 of the fusion protein
C – Y, where C403 of the native enzyme is Y629 of the fusion protein
T – A, where T409 of the native enzyme is A635 of the fusion protein
R – H, where R437 of the native enzyme is H663 of the fusion protein
R – K, where R575 of the native enzyme is K801 of the fusion protein
I – T, where I817 of the native enzyme is T1043 of the fusion protein
E – D, where E836 of the native enzyme is D1062 of the fusion protein
M – V, where M847 of the native enzyme is V1073 of the fusion protein
K – R, where K848 of the native enzyme is R1074 of the fusion protein

Protease cleavage Thrombin (LVPRGS) residues 221 - 226

Cloning sites *Bam*H1 and *Bgl*II of pGEX-2T

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Nucleotide sequence of insert

ggatccATGCCGGTCCGGAAGCAAGATACCCAGAGAGCATTGCATCTG
TTGGAAGAATATCGGTTCGAAACTAAaCCAAACCGAAGACAGACAGCTC
AGGAGTTCCATAGAGCGGGTTATTAGCATATTTTCAGAGCAACCTCTTT
CAGGCTTTAATAGACATTCAAGAATTTTATGAAGTGACCTTACTTGAT
AATCCAAAATGTGTGGATCATTCAAAGCAGTGTGAACCGGTCCAACCC
GGGAATCCTTGGGAGAGTGGCAGCCTTTCAAGTGCTGCTGTGACTTCA
GAGAGCCTGCCCCGGCGGCCTTAGCCCTCCAGTAGAGAAATACCGGTAT
CAGGACGAAGAGGTGCTGCCTTCAGAGCGGATTTCCCCGCAAGTCCCA
AATGAGGTGCTGGGTCCGGAGCTGGTTCACGTCTCGGAGAAGAGCCTG
TCAGAGATTGAGAATGTCCACGGGTTTGTCTCTCACTCTCATATCTCA
CCCATAAAGGCAAATCCTCCTCCTGTGCTGGTCAACACAGACAGCTTG
GAGACACCAACTTATGTTAATGGTACTGATGCAGATTATGAATATGAG
GAAATCACACTTGAAAGGGGAAATTCAGGCCTTGGTTTCAGCATTGCA
GGAGGTACAGACAACCCACACATTGGAGATGACTCAAGTATTTTCATC
ACCAAAATATCACGGGAGGAGCAGCTGCCAGGATGGAAGATTGCGG
GTAAATGACTGTATACTGAGAGTAAATGAAGCAGATGTTTCGTGATGTA
ACCCACAGCAAAGCAGTTGAAGCATTGAAAGAAGCTGGATCGATAGTG
CGATTATATGTGAAAAGGCGGAAGCCAGCCTCAGAAAAAATCATGGAA
ATAAACTTATTAAAGGCCCTAAAGGTCTTGGGTTTCAGCATTGCTGGA
GGTGTGGAAACCAGCACATTCTTGGGGATAACAGCATCTATGTAACC
AAAATAATTGAAGGAGGTGCAGCACACAAGGACGGCAAACCTTCAGATT
GGAGACAAGCTTCTAGCAGTGAACAGTGTCTGTTTAGAAGAAGTTACT
CATGAAGAAGCAGTGACTGCCTTAAAGAATACATCTGATTTTGTTTAT
TTGAAAGTGGCAAAACCAACAAGTATGTATATAAATGATGGCTATGCA
CCACCCGACATCACTAATTCTTCTTCTCAGTCTGTTGACAACCATGTT
AGCCCGTCTTCATACTTGGGCCAGACTCCAGCGTCACCAGCCAGATAAC
TCACCCATTTCTAAAGCAGTGCTTGGAGATGATGAGATCACTAGGGAA
CCTAGAAAAGTTGTTCTTCATCGTGGCTCAACGGGACTTGGTTTCAAC
ATTGTGGGAGGCGAAGATGGAGAAGGGATTTTTATCTCTTTCATCCTT
GCTGGGGACCTGCTGACCTAAGCGGAGAGCTCAGAAAAGGAGATCGC
ATTATATCGGTAAACAGTGTGACCTCAgAGCTGCAAGTCATGAACAA
GCAGCTGCCGCATTAAAAACGCAGGCCAAGCTGTCACTATTGTGCGCA
CAGTATCGGCCTGAAGgAGTACAGTCGTTTTGAAGCTAAAATTCACGAC
TTGCGGGAGCAGATGATGAACAGCAGTGTGAGCTCAGGGTCAGGGTCT
CTCCGAACCAGCCAAAAGCGGTCCCTCTATGTCAGAGCCCTTTTTGAT
TACGACAAGACTAAAGACAGTGGCCTTCCAGTCAAGGGTTGAACTTC
AAATTCGGAGATATTCTCCATGTTATCAATGCTTCTGATGACGAGTGG
TGGCAGGCCAGACAGGTGACCCAGATGGTGAAGTGAAGTGGAGTGGG
GTAATTCCTAGTAAACGCAGAGTTGAGAAGAAAGAACGAGCCCGATTA
AAAACAGTCAAATTC AATTCTAAAACAAGAGGAGATAAAGGGTCATTC
AATGACAAGCGTAAAAAGAACCTCTTTTCCCGAAAATTTCCCTTCTAC
AAGAACAAGGACCAGAGTGAGCAGGAAACAAGTATGCTGACCAGCAC
GTAATTCCTAATGCCAGCGATAGCGAAAGTAGTTACCGTGGTCAAGAA
GAATATGTTTTATCGTATGAGCCGGTGAATCAGCAAGAAGTTAATTAT
ACCCGACCAGTCATCATATTAGGACCTATGAAAGACAGAGTAAATGAT
GACTTAATCTCAGAATTTCTGACAAATTTGGATCCTGTGTCCCTCAT
ACAAC TAGACCAAAGCGTGACTATGAGGTGGATGGACGAGATTACCAT

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TTTGTGACTTCAAGAGAACAGATGGAGAAAGATATCCAGGAGCATAAA
TTCATTGAAGCTGGCCAGTATAACAACCATCTATACGGAACGAGTGTC
CAATCTGTGCGAGCAGTAGCAGAGAAGGGCAAGCATTGTATCCTTGAT
GTGTCTGGAAATGCCATCAAGAGGTTGCAGATTGCACAGCTCTATCCA
ATATCTAcTTTTATTAAACCCAAATCCATGGAAAATATCATGGAAATG
AATAAACGCCTAACAGACGAACAGGCCAGAAAAACGTTTGAGAGAGCA
GTGAGACTGGAGCAGGAGTTCACTGAGCATTTCACAGCTATTGTCCAG
GGAGACACGCTGGAGGACATTTACAATCAAGTGAAGCAGATCATAGAA
GAGCAGTCTGGTCCTTACATCTGGGTCCCAGCAAAAGAAAAATTatga
agatct