Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of VASP [2 - 380]

Enzyme description:- VASP [2 – 380]

Clone number:- DU 1550

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST + HA tag

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 67, 867.26 daltons
Average Mass 67, 910.23 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.60

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

VASP [2 – 380]

Protein VASP [2 – 380]

Clone number DU 1550

Species Human

Accession number P50552.3

Tags N-terminal GST + HA

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSAT*MYPYDVPDY***ASE**
TVICSSRATVMLYDDGNKRWLPAGTGPQAFSRVQIYHNPTANSFRVVGR
KMQPDQQVVINCAIVRGVKYNQATPNFHQWRDARQVWGLNFGSKEDAAQ
FAAGMASALEALEGGGPPPPPALPTWSVPNGPSPEEVEQQKRQQPGPSE
HIERRVSNAGGPPAPPAGGPPPPPGPPPPPGPPPPPGLPPSGVPAAAHG
AGGGPPPAPPLPAAQGPGGGGAGAPGLAAAIAGAKLRKVSKQEEASGGP
TAPKAESGRSGGGGLMEEMNAMLARRRKATQVGEKTPKDESANQEEPEA
RVPAQSESVRRPWEKNSTTLPRMKSSSSVTTSETQPCTPSSSDYSDLQR
VKQELLEEVKKELQKVKEEIIEAFVQELRKRGSP

Native sequence Amino acids A2 – P380 (end) of human VASP.
Residue A243 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220 and the HA tag [*YPYDVPDYA*] is located at residues 235 – 243.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 sites of pGEX6P-1

Nucleotide Sequence of Insert:

gcggatcCGCCACCATGTACCCATACGATGTGCCAGATTACGCCAGCGAG
ACGGTCATCTGTTCCAGCCGGGCCACTGTGATGCTTTATGATGATGGCAA
CAAGCGATGGCTCCCTGCTGGCACGGGTCCCCAGGCCTTCAGCCGCGTCC
AGATCTACCACAACCCCACGGCCAATTCCTTTCGCGTCGTGGGCCGGAAG
ATGCAGCCCGACCAGCAGGTGGTCATCAACTGTGCCATCGTCCGGGGTGT
CAAGTATAACCAGGCCACCCCCAACTTCCATCAGTGGCGCGACGCTCGCC
AGGTCTGGGGCCTCAACTTCGGCAGCAAGGAGGATGCGGCCCAGTTTGCC
GCCGGCATGGCCAGTGCCCTAGAGGCGTTGGAAGGAGGTGGGCCCCCTCC
ACCCCCAGCACTTCCCACCTGGTCGGTCCCGAACGGCCCCTCCCCGGAGG
AGGTGGAGCAGCAGAAAAGGCAGCAGCCCGGCCCGTCGGAGCACATAGAG
CGCCGGGTCTCCAATGCAGGAGGCCCACCTGCTCCCCCCGCTGGGGGTCC
ACCCCCACCACCAGGACCTCCCCCTCCTCCAGGTCCCCCCCCCACCCCCAG
GTTTGCCCCCTTCGGGGGTCCCAGCTGCAGCGCACGGAGCAGGGGGAGGA
CCACCCCCTGCACCCCCTCTCCCGGCAGCACAGGGCCCTGGTGGTGGGGG
AGCTGGGGCCCCAGGCCTGGCCGCAGCTATTGCTGGAGCCAAACTCAGGA
AAGTCAGCAAGCAGGAGGAGGCCTCAGGGGGGCCCACAGCCCCCAAAGCT
GAGAGTGGTCGAAGCGGAGGTGGGGGACTCATGGAAGAGATGAACGCCAT
GCTGGCCCGGAGAAGGAAAGCCACGCAAGTTGGGGAGAAAACCCCCAAGG
ATGAATCTGCCAATCAGGAGGAGCCAGAGGCCAGAGTCCCGGCCCAGAGT
GAATCTGTGCGGAGACCCTGGGAGAAGAACAGCACAACCTTGCCAAGGAT
GAAGTCGTCTTCTTCGGTGACCACTTCCGAGACCCAACCCTGCACGCCCA
GCTCCAGTGATTACTCGGACCTACAGAGGGTGAAACAGGAGCTTCTGGAA
GAGGTGAAGAAGGAATTGCAGAAAGTGAAAGAGGAAATCATTGAAGCCTT
CGTCCAGGAGCTGAGGAAGCGGGGTTCTCCCtgaggatcc

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