Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of AMSH-LP [1 - 436]

Enzyme description:- AMSH-LP [1 – 436]

Clone number:- DU 5601

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 77, 608.60 daltons
Average Mass 77, 658.47 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.39

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

AMSH-LP [1 – 436]

Protein AMSH-LP [1 – 436]

Clone number DU 5601

Species Human

Accession number NM_020799.3

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEIPGSTRAAA**MDQ**
PFTVNSLKKLAAMPDHTDVSLSPEERVRALSKLGCNITISEDITPRRYF
RSGVEMERMASVYLEEGNLENAFVLYNKFITLFVEKLPNHRDYQQCAVP
EKQDIMKKLKEIAFPRTDELKNDLLKKYNVEYQEYLQSKNKYKAEILKK
LEHQRLIEAERKRIAQMRQQQLESEQFLFFEDQLKKQELARGQMRSQQT
SGLSEQIDGSALSCFSTHQNNSLLNVFADQPNKSDATNYASHSPPVNRA
LTPAATLSAVQNLVVEGLRCVVLPEDLCHKFLQLAESNTVRGIETCGIL
CGKLTHNEFTITHVIVPKQSAGPDYCDMENVEELFNVQDQHDLLTLGWI
HTHPTQTAFLSSVDLHTHCSYQLMLPEAIAIVCSPKHKDTGIFRLTNAG
MLEVSACKKKGFHPHTKEPRLFSICKHVLVKDIKIIVLDLR

Native sequence Amino acids M1 – R436 (end) of human AMSH.
Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Not*1 sites of pGEX6P-2

Nucleotide Sequence of Insert:

gcggccgcgATGGATCAGCCTTTTACTGTGAATTCTCTGAAAAAGTTAGC
TGCTATGCCTGACCATACAGATGTTTCCCTAAGCCCAGAAGAGCGAGTCC
GTGCCCTAAGCAAGCTTGGTTGTAATATCACCATCAGTGAAGACATCACT
CCACGACGTTACTTTAGGTCTGGAGTAGAGATGGAGAGGATGGCGTCTGT
GTATTTGGAAGAAGGAAATTTGGAAAATGCCTTTGTTCTTTATAATAAAT
TTATAACCTTATTTGTAGAAAAGCTTCCTAACCATCGAGATTACCAGCAA
TGTGCAGTACCTGAAAAGCAGGATATTATGAAGAAACTGAAGGAGATTGC
ATTCCCAAGGACAGATGAATTGAAAAACGACCTTTTAAAGAAATATAACG
TAGAATACCAAGAATATTTGCAAAGCAAAAACAAATATAAAGCTGAAATT
CTCAAAAAATTGGAGCATCAGAGATTGATAGAGGCAGAAAGGAAGCGGAT
TGCTCAGATGCGCCAGCAGCAGCTAGAATCGGAGCAGTTTCTGTTTTTCG
AAGATCAACTCAAGAAGCAAGAGTTAGCCCGAGGTCAAATGCGAAGTCAG
CAAACCTCAGGGCTGTCAGAGCAGATTGATGGGAGCGCTTTGTCCTGCTT
TTCCACACACCAGAACAATTCCTTGCTGAATGTATTTGCAGATCAACCTA
ATAAAAGTGATGCAACCAATTATGCTAGCCACTCTCCTCCTGTAAACAGG
GCCTTAACACCAGCTGCTACTCTAAGTGCTGTTCAGAATTTAGTGGTTGA
AGGACTGCGATGTGTAGTTTTGCCAGAAGATCTTTGCCACAAATTTCTGC
AACTGGCAGAATCTAATACAGTGAGAGGAATAGAAACCTGTGGAATACTC
TGTGGAAAACTGACACATAATGAATTTACTATTACCCATGTAATTGTGCC
AAAGCAGTCTGCGGGACCAGACTATTGTGACATGGAGAATGTAGAGGAAT
TATTCAATGTTCAGGATCAACATGATCTCCTCACTCTAGGATGGATCCAT
ACACATCCCACTCAAACTGCATTTTTATCCAGCGTTGATCTTCACACTCA
CTGTTCCTATCAACTCATGTTGCCAGAGGCCATTGCCATTGTTTGCTCAC
CAAAGCATAAAGACACTGGCATCTTCAGGCTCACCAATGCTGGCATGCTT
GAGGTTTCTGCTTGTAAAAAAAAGGGCTTTCATCCACACACCAAGGAGCC
CAGGCTGTTCAGTATATGCAAACATGTGTTGGTAAAAGACATAAAAATAA
TTGTGTTGGATCTGAGGtgagcggccgc

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