Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of AMSH [2 - 424]

Enzyme description:- AMSH [2 – 424]

Clone number:- DU 44746

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic	74, 722.15 daltons
Average Mass	74, 769.86 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.84

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 [2 – 424]

Protein	AMSH [2 – 424]
Clone number	DU 44746
Species	Human
Accession number	NM_006463.4
Tags	N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**SDHGDVSLPPEDRV**
RALSQLGSAVEVNEDIPPRRYFRSGVEIIRMASIYSEEGNIEHAFILYN
KYITLFIEKLPKHRDYKSAVIPEKKDTVKKLKEIAFPKAEELKAELLKR
YTKEYTEYNEEKKKEAEELARNMAIQQELEKEKQRVAQQKQQQLEQEQF
HAFEEMIRNQELEKERLKIVQEFGKVDPGLGGPLVPDLEKPSLDVFPTL
TVSSIQPSDCHTTVRPAKPPVVDRSLKPGALSNSESIPTIDGLRHVVVP
GRLCPQFLQLASANTARGVETCGILCGKLMRNEFTITHVLIPKQSAGSD
YCNTENEEELFLIQDQQGLITLGWIHTHPTQTAFLSSVDLHTHCSYQMM
LPESVAIVCSPKFQETGFFKLTDHGLEEISSCRQKGFHPHSKDPPLFCS
CSHVTVVDRAVTITDLR

Native sequence

Amino acids S2 – R424 (end) of human AMSH.
Residue S232 of the fusion protein is equivalent to S2 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage

PreScission (LEVLFQGP) residues 221 - 228

Cloning sites

*Bam*H1 and *Sma*1 sites of pGEX6P-1

Nucleotide Sequence of Insert:

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agatctTCTGACCATGGAGATGTGAGCCTCCCGCCCGAAGACCGGGTGAGGGCTCTCTCCCAGCTG
GGTAGTGCGGTAGAGGTGAATGAAGACATTCCACCCCGTCGGTACTTCCGCTCTGGAGTTGAGATT
ATCCGAATGGCATCCATTTACTCTGAGGAAGGCAACATTGAACATGCCTTCATCCTCTATAACAAG
TATATCACGCTCTTTATTGAGAAACTACCAAAACATCGAGATTACAAATCTGCTGTCATTCCTGAA
AAGAAAGACACAGTAAAGAAATTAAAGGAGATTGCATTTCCCAAAGCAGAAGAGCTGAAGGCAGAG
CTGTTAAAACGATATACCAAAGAATATACAGAATATAATGAAGAAAAGAAGAAGGAAGCAGAGGAA
TTGGCCCGGAACATGGCCATCCAGCAAGAGCTGGAAAAGGAAAAACAGAGGGTAGCACAACAGAAG
CAGCAGCAATTGGAACAGGAACAGTTCCATGCCTTCGAGGAGATGATCCGGAACCAGGAGCTAGAA
AAAGAGCGACTGAAAATTGTACAGGAGTTTGGGAAGGTAGACCCTGGCCTAGGTGGCCCGCTAGTG
CCTGACTTGGAGAAGCCCTCCTTAGATGTGTTCCCCACCTTAACAGTCTCATCCATACAGCCTTCA
GACTGTCACACAACTGTAAGGCCAGCTAAGCCACCTGTGGTGGACAGGTCCTTGAAACCTGGAGCA
CTGAGCAACTCAGAAAGTATTCCCACAATCGATGGATTGCGCCATGTGGTGGTGCCTGGGCGGCTG
TGCCCACAGTTTCTCCAGTTAGCCAGTGCCAACACTGCCCGGGGAGTGGAGACATGTGGAATTCTC
TGTGGAAAACTGATGAGGAATGAATTTACCATTACCCATGTTCTCATCCCCAAGCAAAGTGCTGGG
TCTGATTACTGCAACACAGAGAACGAAGAAGAACTTTTCCTCATACAGGATCAGCAGGGCCTCATC
ACACTGGGCTGGATTCATACTCACCCCACACAGACCGCGTTTCTCTCCAGTGTCGACCTACACACT
CACTGCTCTTACCAGATGATGTTGCCAGAGTCAGTAGCCATTGTTTGCTCCCCCAAGTTCCAGGAA
ACTGGATTCTTTAAACTAACTGACCATGGACTAGAGGAGATTTCTTCCTGTCGCCAGAAAGGATTT
CATCCACACAGCAAGGATCCACCTCTGTTCTGTAGCTGCAGCCACGTGACTGTTGTGGACAGAGCA
GTGACCATCACAGACCTTCGAtgacccggg
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