Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MET [956 - 1390]

Enzyme description:- MET [956 – 1390]

Clone number:- DU 35382

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 75, 805.45 daltons
Average Mass 75, 854.21 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.21

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
GGMEDIYFEFMGGKKK Final concentration: 300 uM

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Clone Data Sheet

MET [956 - 1390]

Protein	MET [956 - 1390]
Clone number	DU 35382
Species	Human
Accession number	NM_000245.3
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**KKRKQIKDL** **GSELVRYDARVHTPHLDRLVSARSVSPTTEMVSNESVDYRATFPEDQF** **PNSSQNGSCRQVQYPLTDMSPILTSGDSDISSPLLQNTVHIDLSALNP** **ELVQAVQHVVIGPSSLIVHFNEVIGRGHFGCVYHGTLLDNDGKKIHCA** **VKSLNRITDIGEVSQFLTEGIIMKDFSHPNVLSLLGICLRSEGSPLVV** **LPYMKHGDLRNFIRNETHNPTVKDLIGFGLQVAKGMKYLASKKFVHRD** **LAARNCMLDEKFTVKVADFGLARDMYDKEYYSVHNKTGAKLPVKWMAL** **ESLQTQKFTTKSDVWSFGVLLWELMTRGAPPYPDVNTFDITVYLLQGR** **RLLQPEYCPDPLYEVMLKCWHPKAEMRPSFSELVSRISAIFSTFIGEH** **YVHVNATYVNVKCVAPYPSLLSSEDNADDEVDTRPASFWETS**
Native sequence	Amino acids K956 – S1390 (end) of human MET. Residue K232 of the fusion protein is equivalent to K956 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pFastBac GST 6P1

Nucleotide sequence of insert

ggatccAAAAAGAGAAAGCAAATTAAAGATCTGGGCAGTGAATTAGTT
CGCTACGATGCAAGAGTACACACTCCTCATTTGGATAGGCTTGTAAGT
GCCCGAAGTGTAAGCCCAACTACAGAAATGGTTTCAAATGAATCTGTA
GACTACCGAGCTACTTTTCCAGAAGATCAGTTTCCTAATTCATCTCAG
AACGGTTCATGCCGACAAGTGCAGTATCCTCTGACAGACATGTCCCCC
ATCCTAACTAGTGGGGACTCTGATATATCCAGTCCATTACTGCAAAAT
ACTGTCCACATTGACCTCAGTGCTCTAAATCCAGAGCTGGTCCAGGCA
GTGCAGCATGTAGTGATTGGGCCCAGTAGCCTGATTGTGCATTTCAAT
GAAGTCATAGGAAGAGGGCATTTTGGTTGTGTATATCATGGGACTTTG
TTGGACAATGATGGCAAGAAAATTCACTGTGCTGTGAAATCCTTGAAC
AGAATCACTGACATAGGAGAAGTTTCCCAATTTCTGACCGAGGGAATC
ATCATGAAAGATTTTAGTCATCCCAATGTCCTCTCGCTCCTGGGAATC
TGCCTGCGAAGTGAAGGGTCTCCGCTGGTGGTCCTACCATACATGAAA
CATGGAGATCTTCGAAATTTCATTCGAAATGAGACTCATAATCCAACT
GTAAAAGATCTTATTGGCTTTGGTCTTCAAGTAGCCAAAGGCATGAAA
TATCTTGCAAGCAAAAAGTTTGTCCACAGAGACTTGGCTGCAAGAAAC
TGTATGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTT
GCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAAACA
GGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACT
CAAAAGTTTACCACCAAGTCAGATGTGTGGTCCTTTGGCGTGCTCCTC
TGGGAGCTGATGACAAGAGGAGCCCCACCTTATCCTGACGTAAACACC
TTTGATATAACTGTTTACTTGTTGCAAGGGAGAAGACTCCTACAACCC
GAATACTGCCCAGACCCCTTATATGAAGTAATGCTAAAATGCTGGCAC
CCTAAAGCCGAAATGCGCCCATCCTTTTCTGAACTGGTGTCCCGGATA
TCAGCGATCTTCTCTACTTTCATTGGGGAGCACTATGTCCATGTGAAC
GCTACTTATGTGAACGTAAAATGTGTCGCTCCGTATCCTTCTCTGTTG
TCATCAGAAGATAACGCTGATGATGAGGTGGACACACGACCAGCCTCC
TTCTGGGAGACATCAtaggcggccgc