Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active MET [1051 - 1349]

Enzyme description:-	MET [1051 – 1349]
Clone number:-	DU 61348
Source:-	Recombinant
Expression system:-	Baculovirus expression vector system
Tag:-	N-terminal GST
Purification method:-	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	60, 683.10 daltons
Average Mass	60, 722.54 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:-	6.68
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 [1051 - 1349]

Protein	MET [956 - 1349]
Clone number	DU 61348
Species	Human
Accession number	NM_000245.4
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**VHIDLSALN** **PELVQAVQHVVIGPSSLIVHFNEVIGRGHFGCVYHGTLLDNDGKKIHC** **AVKSLNRITDIGEVSQFLTEGIIMKDFSHPNVLSLLGICLRSEGSPLV** **VLPYMKHGDLRNFIRNETHNPTVKDLIGFGLQVAKGMKYLASKKFVHR** **DLAARNCMLDEKFTVKVADFGLARDMYDKEYYSVHNKTGAKLPVKWMA** **LESLQTQKFTTKSDVWSFGVLLWELMTRGAPPYPDVNTFDITVYLLQG** **RRLLQPEYCPDPLYEVMLKCWHPKAEMRPSFSELVSRISAIFSTFIGE** **HY**
Native sequence	Amino acids V1051– Y1390 (end) of human MET. Residue V232 of the fusion protein is equivalent to V1051 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

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ggatccGTCCACATTGACCTCAGTGCTCTAAATCCAGAGCTGGTCCAG
GCAGTGCAGCATGTAGTGATTGGGCCCAGTAGCCTGATTGTGCATTTC
AATGAAGTCATAGGAAGAGGGCATTTTGGTTGTGTATATCATGGGACT
TTGTTGGACAATGATGGCAAGAAAATTCACTGTGCTGTGAAATCCTTG
AACAGAATCACTGACATAGGAGAAGTTTCCCAATTTCTGACCGAGGGA
ATCATCATGAAAGATTTTAGTCATCCCAATGTCCTCTCGCTCCTGGGA
ATCTGCCTGCGAAGTGAAGGGTCTCCGCTGGTGGTCCTACCATACATG
AAACATGGAGATCTTCGAAATTTCATTCGAAATGAGACTCATAATCCA
ACTGTAAAAGATCTTATTGGCTTTGGTCTTCAAGTAGCCAAAGGCATG
AAATATCTTGCAAGCAAAAAGTTTGTCCACAGAGACTTGGCTGCAAGA
AACTGTATGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGT
CTTGCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAA
ACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAA
ACTCAAAAGTTTACCACCAAGTCAGATGTGTGGTCCTTTGGCGTGCTC
CTCTGGGAGCTGATGACAAGAGGAGCCCCACCTTATCCTGACGTAAAC
ACCTTTGATATAACTGTTTACTTGTTGCAAGGGAGAAGACTCCTACAA
CCCGAATACTGCCCAGACCCCTTATATGAAGTAATGCTAAAATGCTGG
CACCCTAAAGCCGAAATGCGCCCATCCTTTTCTGAACTGGTGTCCCGG
ATATCAGCGATCTTCTCTACTTTCATTGGGGAGCACTATtaggcggcc
gc
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