

Certificate of Analysis

MET D1228H

met proto-oncogene

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0995-0000-1

Lot: 001

Description: Human MET C-terminal fragment, amino acids K₉₅₆-S₁₃₉₀ (as in NCBI/Protein entry NP_000236.2), D1228H mutant, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MET D1228H Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 78,810 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

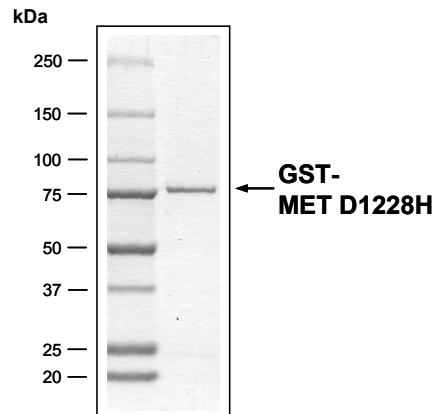
Protein concentration: 0.295 µg/µl

(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

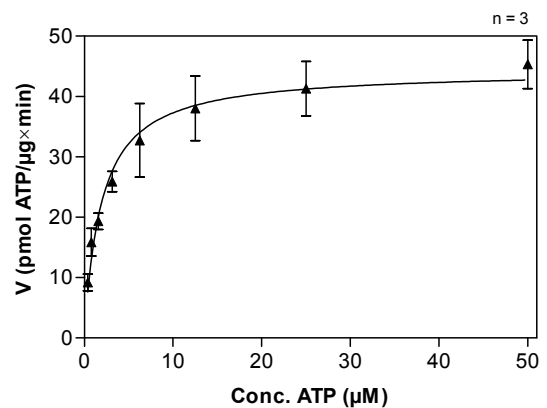
Specific kinase activity (P_i transfer): 44 pmol/µg × min
ATP-K_M: 1.9 µM

MET D1228H Lot 001: Coomassie stain



2.0 µg GST-MET D1228H

MET D1228H Lot 001: Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:

- Assay conditions:
 - 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg/ml PEG_{20,000}
 - ATP (variable)
 - Substrate: TRK-C derived peptide 20 µg/ml
 - Kinase: 1.0 µg/ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: MET D1228H Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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MET D1228H Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYYY	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTSQMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRGS PGLD	GICSIEEFKK	RKQIKDLGSE	LVRVDARVHT	PHLDRLVSAR	SVSPTTEMVS	300
301	NESVDYRATF	PEDQFPNSSQ	NGSCRQVQYP	LTDMSPI LTS	GSDDISSPLL	QNTVHIDL SA	360
361	LNPELVQAVQ	HVIGPSSLI	VHFNEVIGRG	HFGCVYHGTL	LDNDGKKIHC	AVKSLNRITD	420
421	IGEVSQFLTE	GIIMKDFSHP	NVLSLLGICL	RSEGSPLVVL	PYMKHGDLRN	FIRNETHNPT	480
481	VKDLIGFGLQ	VAKGMKYLAS	KKFVHRDLAA	RNCMLDEKFT	VKVADFGLAR	IMYDKEYYSV	540
541	HNKTGAKLPV	KWMALESLOT	QKFTTKSDVW	SFGVLLWELM	TRGAPPYPDV	NTFDITVYLL	600
600	QGRLLQPEY	CPDPLYEVML	KCWHPKAEMR	PSFSELVSRI	SAIFSTFIGE	HYVHV NATYV	660
661	NVKCVAPYPS	LLSSEDNADD	EVDTRPASFW	ETS			720

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** MET fragment **boxed:** D1228H

MET wt ¹ Amino Acid Sequence							
1	MKPAVAVLAPG	ILVLLFTLVQ	RSNGECKEAL	AKSEMNVNMK	YQLPNFTAET	PIQNVLHHEH	60
61	HIFLGATNYI	YVLNEEDLQK	VAEYKTGPVL	EHPDCFPQD	CSSKANLSGG	VWKDNI NMAL	120
121	VVDYYDDQL	ISCGSVNRGT	CQRHVFPNH	TADIQSEVHC	IFSPQIEEPS	QCPDCVVSAL	180
181	GAKVLSSVKD	RFINFFVGNT	INSSYFPDHP	LHSISVRLK	ETKDGFMFLT	DQSYIDVLPE	240
241	FRDSYPIKYV	HAFESNNFIY	FLTQVRETL	AQTFHTRIIR	FCSINSLGHS	YMEMPLCIL	300
301	TEKRKRSTK	KEVFNIIQAA	YVSKPGAQLA	RQIGASLNDD	ILFGVFAQSK	PDSAEPMDRS	360
361	AMCAFPIKYV	NDFFNKIVNK	NNVRCLQHFY	GNHEHCENR	TLLRNSSGCE	ARRDEYRTEF	420
421	TTALQRVDLF	MGQFSEVLLT	SISTFIKGD	TIANLGTSEG	RFMQVVVRS	GPSTPHVNFL	480
481	LDSHPVSPEV	IVEHTLNQNG	YTLVITGKKI	TKIPLNGLGC	RHFQSCSQCL	SAPPFVQCGW	540
541	CHDKCVRSEE	CLSGTWTQQI	CLPAIYKVP	NSAPLEGGTR	LTICGWDFGF	RRNKNFDLKK	600
600	TRVLLGNESC	TLTLESTMN	TLKCTVGPAM	NKHFNM SIII	SNGHGTTQYS	TFSYVDPVIT	660
661	SISPKYGPMA	GGTLLTLTGN	YLN SGN SRHI	SIGGKTCTLK	SVSNSILECY	TPAQTISTEF	720
721	AVKLIKIDLAN	RETSIFS YRE	DPIVYEIHPT	KSFISGGSTI	TGVGKNLNSV	SVPRMIVNH	780
781	EAGRNFTVAC	QHRSNSEIIC	CTTPSLQQLN	LQLPLKTKAF	FMLDGILSKY	FDLIYVHNPV	840
841	FKPFEKPVMI	SMGNENVLEI	KGNDIDPEAV	KGEVLKVGNK	SCENIHLHSE	AVLCTVPNDL	900
901	LKLNSELNIE	WKQAISSTVL	GKVIVQPDQN	FTGLIAGVVS	ISTALLLLLG	FFLWL KKRKQ	960
961	IKDLGSELVR	YDARVHTPHL	DRLVSARSVS	PTTEMVSNES	VDYRATFPED	QFPNSSQNGS	1020
1021	CRQVQYPLTD	MSPILTSGDS	DISSPLLQNT	VHIDL SALNP	ELVQAVQHVV	IGPSSLIVHF	1080
1081	NEVIGRGHFG	CVYHGTL L DN	DGKKIHCAVK	SLNRITDIGE	VSQFLTEGII	MKDFSHPNVL	1140
1141	SLLGICLRSE	GSPLVLPY M	KHGDLRNFIR	NETHNPTVKD	LIGFGLQVAK	GMKYLASKKF	1200
1201	VHRDLAARNC	MLDEKFTVKV	ADFGLARDMY	DKEYYSVH NK	TGAKLPVKWM	ALESLOTQKF	1260
1261	TTKSDVWSFG	VLLWELMTRG	APPYPDVNTF	DITVYLLQGR	RLLOPEYCPD	PLYEVMLKCW	1320
1321	HPKAEMRPSF	SELVSRISAI	FSTFIGEHYV	HVNATYVNVK	CVAPYPSLLS	SEDNADDEVD	1380
1381	TRPASFWETS						1440

blue: MET sequence expressed in fusion protein **Red:** variant in fusion protein

¹NCBI/Protein accession number NP_000236.2

Please notice:

Variant amino acid counting starts with Ser755 when referring to GenBank accession J02958 (additional 18 aa exon between S755/G756, frequently found in the literature)