

ProQinase™ 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

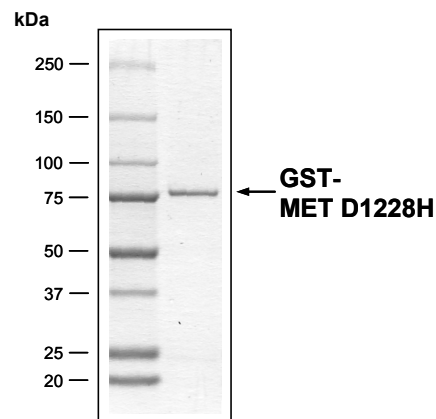
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.295 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

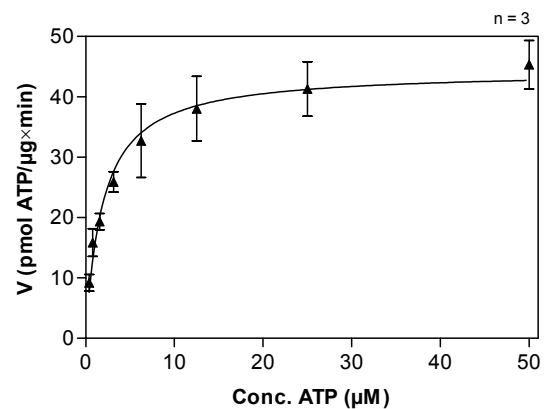
Specific kinase activity (P_i transfer): 44 pmol/µg × min
ATP-K_M: 1.9 µM

**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 Reaction Biology for the use with the ADP-Glo™ Kinase assay from ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



ProQinase™ MET D1228H

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MET D1228H Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSIEEFKK	RKQIKDLGSE	LVRVDARVHT	PHLDRLVSAR	SVSPTTEMVS	300
301	NEVDYRATF	PEDQFPNSSQ	NGSCRQVQYP	LTDMSPIILTS	GSDDISSPLL	QNTVHIDLSA	360
361	LNPELVQAVQ	HVIGPSSLI	VHFNEVIGRG	HFGCVYHGTL	LDNDGKKIHC	AVKSLNRITD	420
421	IGEVSOFLTE	GIIMKDFSHP	NVLSLLGICL	RSEGSPLVVL	PYMKHGDLRN	FIRNETHNPT	480
481	VKDLIGFGLQ	VAKGMKYLAS	KKFVHRDLAA	RNCMLDEKFT	VKVADFGLAR	HMYDKEYYSV	540
541	HNKTAGLQPV	KWMALESLOT	QKFTTKSDVW	SFGVLLWELM	TRGAPPYDV	NTFDITVYLL	600
600	QGRRLQPEY	CPDPLYEVML	KCWHPKAEMR	PSFSELVSRI	SAIFSTFIGE	HYVHVNATYV	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	MKAPAVLAPG	ILVLLFTLVQ	RSNGECKEAL	AKSEMNVNMK	YQLPNFTAET	PIQNVILHEH	60
61	HIFLGATNYI	YVLNEEDLQK	VAEYKTGPVL	EHPDCFCPCQD	CSSKANLSGG	VWKDINMAL	120
121	VVDYYDDQL	ISCGSVNRGT	CQRHVFPNH	TADIQSEVHC	IFSPQIEEPS	QCPDCVVSAL	180
181	GAKVLSSVKD	RFINFFVGNT	INSSYFPDHP	LHSISVRRLK	ETKDGFMFLT	DQSYIDVLPE	240
241	FRDSYPIKYV	HAFESNNFIY	FLTVQRETLD	AQTFHTRIIR	FCSINSLGHS	YMEMPLCICL	300
301	TEKRKRSTK	KEVFNILQAA	YVSKPGAQLA	RQIGASLNDD	ILFGVFAQSK	PDSAEPMDRS	360
361	AMCAFPKYV	NDFFNKIVNK	NNVRCLQHFY	GNHEHCFNR	TLLRNSSGCE	ARRDEYRTEF	420
421	TTALQRVDLF	MGQFSEVLLT	SISTFIKGD	TIANLGTSEG	RFMQVVVRS	GPSTPHVNFL	480
481	LDSHPVSPEV	IVEHTLNQNG	YTLVITGKKI	TKIPLNGLGC	RHFQSCSQCL	SAPPFVQCGW	540
541	CHDKCVRSEE	CLSGTWTQQI	CLPAIYKVPF	NSAPLEGGTR	LTICGWDFGF	RRNKFDLKK	600
600	TRVLLGNESC	TLTLESTMN	TLKCTVGPAM	NKHFNMSIII	SNGHGTTQYS	TFSYVDPVIT	660
661	SISPKYGPPA	GGTLLTLTGN	YLNNSGNSRHI	SIGGKTCTLK	SVSNSILECY	TPAQTISTEF	720
721	AVKLIKIDLAN	RETSIFSIRE	DPIVYEIHPT	KSFISGGSTI	TGVGKNLNSV	SVPRMIVNH	780
781	EAGRNFVAC	QHRNSNFIIC	CTTPSLQQLN	LQLPLKTKAF	FMLDGILSKY	FDLIYVHNVP	840
841	FKPFEKPVMI	SMGNENVLEI	KGNDIDPEAV	KGEVLKVGNK	SCENIHLHSE	AVLCTVPNDL	900
901	LKLNSELNIE	WKQAISSTVL	GKVIVQPDQN	FTGLIAGVVS	ISTALLLLL	FFLWLKRRKQ	960
961	IKDLGSELVR	YDARVHTPHL	DRLVSARSVS	PTTEMVSNES	VDYRATFPED	QFPNSSQNGS	1020
1021	CRQVQYPLTD	MSPILTSGDS	DISSPLLQNT	VHIDLSALNP	ELVQAVQHVY	IGPSSLIVHF	1080
1081	NEVIGRGHFG	CVYHGTLTLDN	DGKKIHC	CAVKSLNRITDIGE	VSQFLTEGII	MKDFSHPNVL	1140
1141	SLLGICLRSE	GSPLVLPYIM	KHGDLRNFIR	NETHNPTVKD	LIGFGLQVAK	GMKYLASKKF	1200
1201	VHRDLAARNC	MLDEKFTVKV	ADFGLARDMY	DKEYYSVHNK	TGAKLPVKWM	ALESLOTQKF	1260
1261	TTKSDVWSFG	VLLWELMTRG	APPYPDVNTF	DITVYLLQGR	RLLQPEYCPD	PLYEVMLKCW	1320
1321	HPKAEMRPSF	SELVSRISAI	FSTFIGEYHV	HVNATYVNVK	CVAPYPSLLS	SEDNADDEV	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)