

MET D1228N

MET proto-oncogene, receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: MET

Synonyms: c-MET, HGFR

Product No.: 0994-0000-1

Lot: 001

Description: Human MET C-terminal fragment, amino acids K₉₅₆-S₁₃₉₀ (as in [NCBI/Protein](#) entry NP_000236.2), D1228N mutant, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: MET D1228N Lot 001 product identity was confirmed by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 78,787 Da

Expression host: Sf9 insect 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: 0.437 µg/µl

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

Biochemical Parameters:

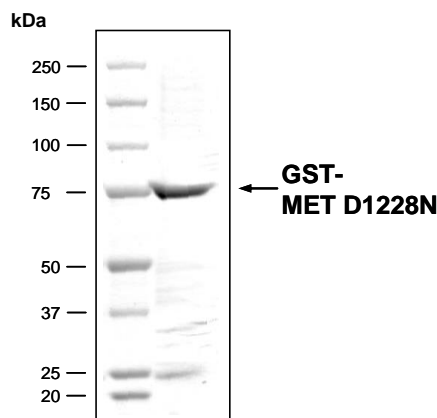
Specific kinase activity (P_i transfer): 59 pmol/µg × min

ATP-K_M: 1.4 µM

Additional assay technology:

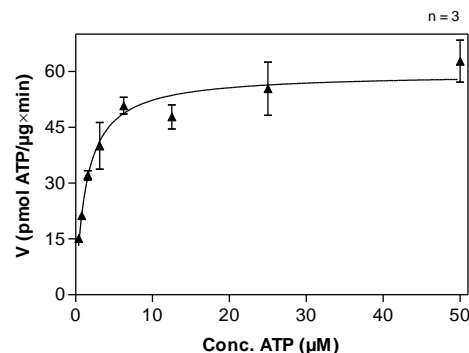
MET D1228N Lot 001 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

MET D1228N Lot 001:
Coomassie stain



2.0 µg GST-MET D1228N

MET D1228N 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 µg/ml

• Filter binding assay

MSPH membrane (Millipore)

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GST-MET D1228N Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHG	RRRASVAAGI	240
241	LVPRGS PGLD	GICSIIEFKK	RKQIKDLGSE	LVRVDARVHT	PHLDRLVSAR	SVSPTTEMVS	300
301	NESVDYRATF	PEDQFPNSSQ	NGSCRQVQYP	LTDMSPILTS	GSDISSPLL	QNTVHIDLSA	360
361	LNPELVQAVQ	HVVIGPSSLI	VHFNEVIGRG	HFGCVYHGTL	LDNDGKKIHC	AVKSLNRTD	420
421	IGEVSQFLTE	GIIMKDFSHP	NVLSLLGICL	RSEGSPLVVL	PYMKHGDLRN	FIRNETHNPT	480
481	VKDLIGFGLQ	VAKGMKYLAS	KKFVHRDLAA	RNCMLDEKFT	VKVADFGLAR	NMYDKEYYSV	540
541	HNKTKAKLPV	KWMALESLOT	QKFTTKSDVW	SFGVLLWELM	TRGAPPYDPV	NTFDITVYLL	600
600	QGRRLLOPEY	CPDPLYEVML	KCWHPKAEMR	PSFSELSVRI	SAIFSTFIGE	HYVHVNTATV	660
661	NVKCVAPYPS	LLSSEDNADD	EVDTRPASFW	ETS			720

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: MET fragment **boxed**: D1228N

MET wt ¹ Amino Acid Sequence							
1	MKAPAVLAPG	ILVLLFTLVQ	RSNGECKEAL	AKSEMNVNMK	YQLPNFTAET	PIQNVILHEH	60
61	HIFLGATNYI	YVLNEEDLQK	VAEYKTGPVL	EHPDCFPQD	CSSKANLSGG	VWKDINMAL	120
121	VVDYYDDQL	ISCGSVNRGT	QQRHVFPNH	TADIQSEVHC	IFSPQIEEPS	QCPDCVSAL	180
181	GAKVLSSVKD	RFINFFVGN	INSSYFPDHP	LHSISVRLK	ETKDGFMFLT	DQSYIDLPE	240
241	FRDSYPIKYV	HAFESNNFY	FLTVQRETL	AQTFHTRIR	FCSINSLHG	YMEMPLECIL	300
301	TEKRKKRSTK	KEVFNILQAA	YVSKPGAQLA	RQIGASLND	ILFGVFAQSK	PDSAEPMDRS	360
361	AMCAFPKIYV	NDFFNKIVNK	NNVRCLQHFY	GNHEHCENR	TLLRNSSGCE	ARRDEYRTEF	420
421	TTALQRVDFL	MGQFSEVLLT	SISTFIKGD	TIANLGTSEG	RFMQVVVSR	GPSTPHVNF	480
481	LDSHPVSPEV	IVEHTLNQNG	YTLVITGK	TKIPLNGLG	RHFQSCSQCL	SAPPFVQCGW	540
541	CHDKCVRSEE	CLSGTWTQQI	CLPAIYKVP	NSAPLEGGTR	LTICGWDFGF	RRNNKFDLKK	600
600	TRVLLGNESC	TLTLESTMN	TLKCTVGPAM	NKHFNMSII	SNGHGTQYS	TFSYVDPVIT	660
661	SISPKYGPMA	GGTLLTTLGN	YLNNGNSRHI	SIGGKTCTLK	SVSNSILECY	TPAQTISTEF	720
721	AVKCLKIDLAN	RETSIFSIRE	DPIVYIEIHT	KSFISGGSTI	TGVGKNLSV	SVPRMIVNH	780
781	EAGRNFVAC	QHRNSSEIIC	CTTPSLQQLN	LQLPLKTKAF	FMLDGILSKY	FDLIYVHNPV	840
841	FKPFKPVMI	SMGNENVLEI	KGNDIDPEAV	KGEVLKVGNK	SCENIHLHSE	AVLCTVPNDL	900
901	LKLNSELNIE	WKQAISSVTL	GKVIVQPDQN	FTGLIAGVVS	ISTALLLLLG	FFLWL KRRQ	960
961	IKDLGSELVR	YDARVHTPHL	DRLVSARSVS	PTEMVSNES	VDYRATFPED	QFPNSSQNGS	1020
1021	CRQVQYPLTD	MSPILTSGDS	DISSPLLQNT	VHIDLALNP	ELVQAVQHV	IGPSSLIHF	1080
1081	NEVIGRGHFG	CVYHGTLTLDN	DGKKIHCAVK	SLNRITDIGE	VSQFLTEGII	MKDFSHPNVL	1140
1141	SLLGICLRSE	GSPLVLPYM	KHGDLRNFIR	NETHNPTVKD	LIGFGLQVAK	GMKYLASKKE	1200
1201	VHRDLAARNC	MLDEKFTVKV	ADFGLARDMY	DKEYYSVHNC	TGAKLPVKWM	ALESLOTQKF	1260
1261	TTKSDVWSFG	VLLWELMTRG	APPYDPVNTF	DITVYLLQGR	RLLOPEYCPD	PLYEVMLKCW	1320
1321	HPKAEMRPSF	SELVSRISAI	FSTFIGEHYV	HVNATYVNVK	CVAPYPSLLS	SEDNADDEVD	1380
1381	TRPASFWETS						1440

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

¹[NCBI/Protein](#) accession number NP_000236.2

Please notice:

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

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