

## ProQinase™ EGF-R T790M

epidermal growth factor receptor

Recombinant Human Active Protein Kinase

HGNC Symbol: EGFR

Synonyms: ERBB, ERBB1

Product No.: 0758-0000-1

Lot: 001

**Description:** Human EGF-R, C-terminal fragment, amino acids H<sub>672</sub>-A<sub>1210</sub> (as in [NCBI/Protein](#) entry NP\_005219.2), T<sub>790M</sub> mutant, N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** EGF-R T790M Lot 001, was confirmed as EGF-R by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 89,201 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:** 0.171 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

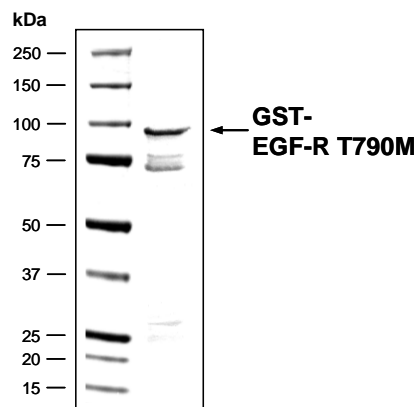
**Biochemical Parameters:**

Specific kinase activity (P<sub>i</sub> transfer): 32 pmol/µg × min  
ATP-K<sub>M</sub>: 0.6 µM

**Additional assay technology:**

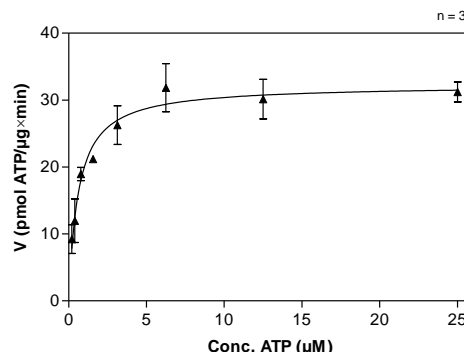
EGF-R T790M Lot 001 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

**EGF-R T790M Lot 001:  
Coomassie stain**



2.0 µg GST-EGF-R T790M

**EGF-R T790M Lot 001:  
Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP**



**Determination of K<sub>M</sub> value & Specific activity:**

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg/ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: Poly(Glu:Tyr)<sub>4:1</sub> 5 µg/ml
  - Kinase: 1 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

## ProQinase™ EGF-R T790M

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GST-EGF-R T790M 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 <b>HHHHHG</b>	RDS <b>LEVLFCG</b>	240
241	<b>PLAMGHIVRK</b>	<b>RTLRLQLQER</b>	<b>ELVEPLTPSG</b>	<b>EAPNQALLRI</b>	<b>LKETEFKKIK</b>	<b>VLGSGAFGTV</b>	300
301	<b>YKGLWIPEGE</b>	<b>KVKIPVAIKE</b>	<b>LREATSPKAN</b>	<b>KEILDEAYVM</b>	<b>ASVDNPHVCR</b>	<b>LLGICLTSTV</b>	360
361	<b>QLIMQLMPFG</b>	<b>CLLDYVREHK</b>	<b>DNIGSQYLLN</b>	<b>WCVQIAKGMN</b>	<b>YLEDRLVHR</b>	<b>DLAARNVLVK</b>	420
421	<b>TPQHVKITDF</b>	<b>GLAKLLGAE</b>	<b>KEYHAEGGKV</b>	<b>PIKWMALESI</b>	<b>LHRIYTHQSD</b>	<b>VWSYGVTVWE</b>	480
481	<b>LMTFGSKPYD</b>	<b>GIPASEISSI</b>	<b>LEKGERLPQP</b>	<b>PICTIDVYMI</b>	<b>MVKCWMIDAD</b>	<b>SRPKFRELI</b>	540
541	<b>EFSKMARDPQ</b>	<b>RYLVIQGD</b>	<b>MHLPSPTDSN</b>	<b>FYRALMDEED</b>	<b>MDDVDADEY</b>	<b>LIPQQGFFSS</b>	600
600	<b>PSTSRTPLLS</b>	<b>SLSATSNNST</b>	<b>VACIDRNLQ</b>	<b>SCPIKEDSFL</b>	<b>QRYSSDPTGA</b>	<b>LTEDSIDDTF</b>	660
661	<b>LPVPEYINQS</b>	<b>VPKRPAGSVQ</b>	<b>NPVYHNQPLN</b>	<b>PAPSRDPHYQ</b>	<b>DPHSTAVGNP</b>	<b>EYLNVTQPTC</b>	720
721	<b>VSNFTDSPA</b>	<b>WAQKGSQIS</b>	<b>LDNPDYQDF</b>	<b>FPKEAKPNGI</b>	<b>FKGSTAENAE</b>	<b>YLRVAPQSE</b>	780
781	<b>FIGA</b>						840

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: EGF-R fragment boxed: T790M mutation

EGF-R wt <sup>1</sup> Amino Acid Sequence							
1	MRPSGTAGAA	LLALLAALCP	ASRALEEKV	COGTSNKLTQ	LGTTFEDHFLS	LQRMFNCEV	60
61	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL	IALNTVERIP	LENLQIIRGN	MYYENSYALA	120
121	VLSNYDANKT	GLKELPMRNL	QEILHGAVRF	SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	180
181	QNHLSGCQKC	DPSCPNGSCW	GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	240
241	TGPRESCLV	CRKFRDEATC	KDTCPPMLY	NPTTYQMDVN	PEGKYSFGAT	CVKKCPRNVV	300
301	VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	INATNIKHFK	360
361	NCTSISGDLH	ILPVAFRGDS	FTHTPPLDPQ	ELDILKTVKE	ITGFLLIQAW	PENRTDLHAF	420
421	ENLEIIRGRT	KQHGQFSLAV	VSLNITSLGL	RSKLEISDGD	VIISGNKNLC	YANTINWKKL	480
481	FGTSGQKTKI	ISNRGENSCK	ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCN	540
541	LLEGEPEFV	ENSECIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
600	GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCPNG	PKIPSIATGM	VGALLLLLVV	660
661	ALGIGLFMR	<b>RHIVRKRTL</b>	<b>RLQERELVE</b>	<b>PLTPSGEAPN</b>	<b>QALLRILKET</b>	<b>EFKKIKVLGS</b>	720
721	<b>GAFGTVYKGL</b>	<b>WIPEGEKVKI</b>	<b>PVAIKELREA</b>	<b>TSPKANKEIL</b>	<b>DEAYVMASVD</b>	<b>NPHVCRLLGI</b>	780
781	<b>CLTSTVQLIT</b>	<b>QLMPFGCLLD</b>	<b>YVREHKDNIG</b>	<b>SOYLLNWCVQ</b>	<b>IAGMNYLED</b>	<b>RRLVHRDLAA</b>	840
841	<b>RNVLVKTPQH</b>	<b>VKITDFGLAK</b>	<b>LLGAEKEYH</b>	<b>AEGGKVPKW</b>	<b>MALESILHRI</b>	<b>YTHQSDVWSY</b>	900
901	<b>GVTVWELMTF</b>	<b>GSKPYDGIPA</b>	<b>SEISSILEKG</b>	<b>ERLPQPPICT</b>	<b>IDVYMIMVKC</b>	<b>WMIDADSRPK</b>	960
961	<b>FRELIIIEFSK</b>	<b>MARDPQRYLV</b>	<b>IQDERMHLP</b>	<b>SPTDSNFYRA</b>	<b>LMDEEDMDV</b>	<b>VDADEYLIPQ</b>	1020
1021	<b>QGFFSSPSTS</b>	<b>RTPLSSLSA</b>	<b>TSNNSTVACI</b>	<b>DRNLQSCPI</b>	<b>KEDSFLQRY</b>	<b>SDPTGALTED</b>	1080
1081	<b>SIDDTFLPVP</b>	<b>EYINQSVPKR</b>	<b>PAGSVQNPVY</b>	<b>HNQPLNPAPS</b>	<b>RDPHYQDPHS</b>	<b>TAVGNPEYLN</b>	1140
1141	<b>TVQPTCVNST</b>	<b>FDSPAHAQK</b>	<b>GSHQISLDNP</b>	<b>DYQDFFPKE</b>	<b>AKPNGIFKGS</b>	<b>TAENAEYLRV</b>	1200
1201	<b>APQSSEFIGA</b>						1260

blue: EGF-R sequence expressed in recombinant protein Red: variant in recombinant protein

<sup>1</sup>NCBI/Protein accession number NP\_005219.2