Certificate of Analysis

REACTION BIOLOGY

ProQinase™ EGF-R G719S

epidermal growth factor receptor

Recombinant Human Active Protein Kinase

HGNC Symbol: EGFR

Synonyms: ERBB, ERBB1, HER1, mENA, PIG61

Product No.: 1035-0000-1

Lot: 001

Description: Human EGF-R, C-terminal fragment, amino acids H_{672}-A_{1210} (as in NCBI/Protein entry NP_005219.2), G_{719}S mutant, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: EGF-R G719S Lot 001, was confirmed as EGF-R by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 89,201 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

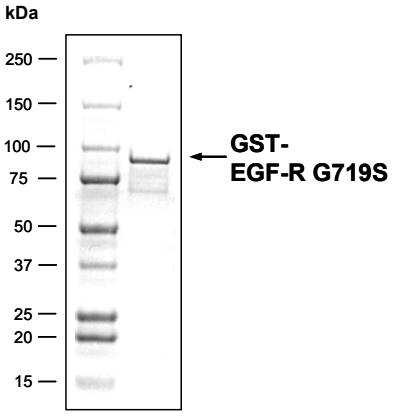
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

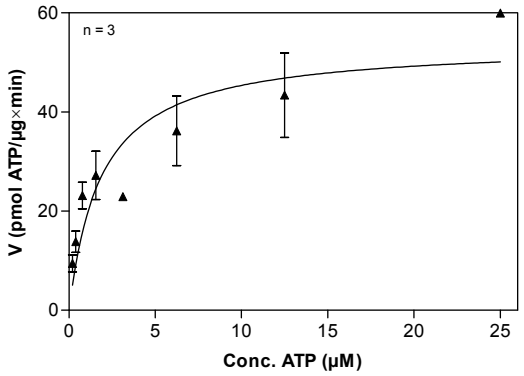
Biochemical Parameters:
Specific kinase activity (P_i transfer): 54 pmol/µg × min
ATP-K_M: 1.9 µM

EGF-R G719S Lot 001:
Coomassie stain



2.0 µg GST-EGF-R G719S

EGF-R G719S 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 $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: Poly(Glu:Tyr)$_{4:1}$ 5.0 µg/ml
  Kinase: 1.0 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology: EGF-R G719S
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

Recombinant Proteins

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ProQinase™ EGF-R G719S

Product No.: 1035-0000-1

Recombinant Proteins

EGF-R G719S Recombinant Fusion Protein Amino Acid Sequence

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
 241 PLAMGHIVRK RTLRRLLQER ELVEPLTPSG EAPNQALLRI LKETEFKKIK VLSSGAFGTV 300
 301 YKGLWIPEGE KVKIPVAIKE LREATSPKAN KEILDEAYVM ASVDNPHVCR LLGICLTSTV 360
 361 QLITQLMPFG CLLDYVREHK DNIGSQYLLN WCVQIAKGMN YLEDRRLVHR DLAARNVLVK 420
 421 TPQHVKITDF GLAKLLGAEE KEYHAEGGKV PIKWMALESI LHRIYTHQSD VWSYGVTVWE 480
 481 LMTFGSKPYD GIPASEISSI LEKGERLPQP PICTIDVYMI MVKCWMIDAD SRPKFRELII 540
 541 EFSKMARDPQ RYLVIQGDER MHLPSPTDSN FYRALMDEED MDDVVDADEY LIPQQGFFSS 600
 600 PSTSRTPLLS SLSATSNNST VACIDRNGLQ SCPIKEDSFL QRYSSDPTGA LTEDSIDDTF 660
 661 LPVPEYINQS VPKRPAGSVQ NPVYHNQPLN PAPSRDPHYQ DPHSTAVGNP EYLNTVQPTC 720
 721 VNSTFDSPAH WAQKGSHQIS LDNPDYQQDF FPKEAKPNGI FKGSTAENAE YLRVAPQSSE 780
 781 FIGA                                                              840
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1-218: GST **Red:** HIS6-tag **Green:** 3C/TEV cleavage site **blue**: EGF-R fragment **boxed**:G719S mutation

EGF-R wt[1] Amino Acid Sequence

```
   1 MRPSGTAGAA LLALLAALCP ASRALEEKKV CQGTSNKLTQ LGTFEDHFLS LQRMFNNCEV  60
  61 VLGNLEITYV QRNYDLSFLK TIQEVAGYVL IALNTVERIP LENLQIIRGN MYYENSYALA 120
 121 VLSNYDANKT GLKELPMRNL QEILHGAVRF SNNPALCNVE SIQWRDIVSS DFLSNMSMDF 180
 181 QNHLGSCQKC DPSCPNGSCW GAGEENCQKL TKIICAQQCS GRCRGKSPSD CCHNQCAAGC 240
 241 TGPRESDCLV CRKFRDEATC KDTCPPLMLY NPTTYQMDVN PEGKYSFGAT CVKKCPRNYV 300
 301 VTDHGSCVRA CGADSYEMEE DGVRKCKKCE GPCRKVCNGI GIGEFKDSLS INATNIKHFK 360
 361 NCTSISGDLH ILPVAFRGDS FTHTPPLDPQ ELDILKTVKE ITGFLLIQAW PENRTDLHAF 420
 421 ENLEIIRGRT KQHGQFSLAV VSLNITSLGL RSLKEISDGD VIISGNKNLC YANTINWKKL 480
 481 FGTSGQKTKI ISNRGENSCK ATGQVCHALC SPEGCWGPEP RDCVSCRNVS RGRECVDKCN 540
 541 LLEGEPREFV ENSECIQCHP ECLPQAMNIT CTGRGPDNCI QCAHYIDGPH CVKTCPAGVM 600
 600 GENNTLVWKY ADAGHVCHLC HPNCTYGCTG PGLEGCPTNG PKIPSIATGM VGALLLLLVV 660
 661 ALGIGLFMRR RHIVRKRTLR RLLQERELVE PLTPSGEAPN QALLRILKET EFKKIKVLGS 720
 721 GAFGTVYKGL WIPEGEKVKI PVAIKELREA TSPKANKEIL DEAYVMASVD NPHVCRLLGI 780
 781 CLTSTVQLIT QLMPFGCLLD YVREHKDNIG SQYLLNWCVQ IAKGMNYLED RRLVHRDLAA 840
 841 RNVLVKTPQH VKITDFGLAK LLGAEEKEYH AEGGKVPIKW MALESILHRI YTHQSDVWSY 900
 901 GVTVWELMTF GSKPYDGIPA SEISSILEKG ERLPQPPICT IDVYMIMVKC WMIDADSRPK 960
 961 FRELIIEFSK MARDPQRYLV IQGDERMHLP SPTDSNFYRA LMDEEDMDDV VDADEYLIPQ 1020
1021 QGFFSSPSTS RTPLLSSLSA TSNNSTVACI DRNGLQSCPI KEDSFLQRYS SDPTGALTED 1080
1081 SIDDTFLPVP EYINQSVPKR PAGSVQNPVY HNQPLNPAPS RDPHYQDPHS TAVGNPEYLN 1140
1141 TVQPTCVNST FDSPAHWAQK GSHQISLDNP DYQQDFFPKE AKPNGIFKGS TAENAEYLRV 1200
1201 APQSSEFIGA                                                        1260
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blue: EGF-R sequence expressed in fusion protein **Red**: variant in fusion protein

[1]NCBI/Protein accession number NP_005219.2