

Certificate of Analysis

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 H₆₇₂-A₁₂₁₀ (as in NCBI/Protein entry NP_005219.2), G₇₁₉S mutant, N-terminal GST-HIS₆ 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:

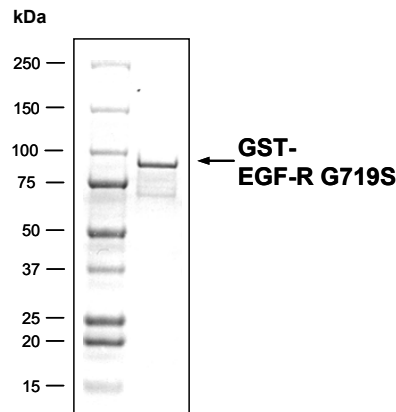
Specific kinase activity (P_i transfer): 54 pmol/µg × min

ATP-K_M: 1.9 µM

Additional assay technology: EGF-R G719S

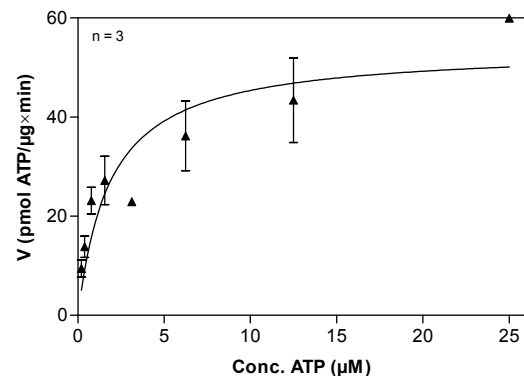
was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

**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 MgCl₂
 - 3 mM MnCl₂
 - 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)

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EGF-R G719S Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYTE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH G	RDS LEVL FQG	240
241	PLAMGHIVRK	RTLRRLLQER	ELVEPLTPSG	EAPNQALLRI	LKETEFKKIK	VL S SGAFGTV	300
301	YKGLWIPEGE	KVKIPVAIKE	LREATSPKAN	KEILDEAYVM	ASVDNPHVCR	LLGICLTSTV	360
361	QLITQLMPFG	CLLDYVREHK	DNIGSQYLLN	WCVQIAKGMN	YLEDRLVHR	DLAARNLVK	420
421	TPQHVKITDF	GLAKLLGAE	KEYHAEGGKV	PIKWMALESI	LHRIYTHQSD	VWSYGVTVWE	480
481	LMTFGSKPYD	GIPASEISSI	LEKGERLPQP	PICTIDVYMI	MVKCWMIDAD	SRPKFRELII	540
541	EFKSMARDPQ	RYLVIQGDER	MHLPSPTDSN	FYRALMDEED	MDDVVDAD EY	LIPQQGFFSS	600
600	PSTSRTPLLS	SLSATSNNST	VACIDRNLQ	SCPIKEDSFL	QRYSSDPTGA	LTEDSIDDTF	660
661	LPVPEYINQS	VPKRPAGSVQ	NPVYHNQPLN	PAPSRDPHYQ	DPHSTAVGNP	EYLNTVQPTC	720
721	VNSTFDSPA H	WAQKGS HQIS	LDNPDYQ QDF	FPKEAKPNGI	FKGSTAENAE	YLRVAPQ SSE	780
781	FIGA						840

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	ASRALEEKVV	CQGTSNKLTQ	LGT FEDHFLS	LQRMFN NCEV	60
61	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL	IALNTVERIP	LENLQIIRGN	MYYENS YALA	120
121	VLSNYDANKT	GLKELPMRNL	QEILHGAVRF	SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	180
181	QNH LGSCQKC	DPSCPNGSCW	GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	240
241	TGPRES DCLV	CRKFRDEATC	KDTC PPLMLY	NPTTYQMDVN	PEGKYSFGAT	CVKKCPRNYV	300
301	VDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	INATNIK HFK	360
361	NCT SISGDLH	ILPVAFRGDS	FTHTPPLDPQ	ELDILKTVKE	ITGFLLIQAW	PENRTDLHAF	420
421	ENLEIIRGRT	KQHGQFSLAV	VSLNITSLGL	RSLKEISDGD	VIISGNKNLC	YANTINWKKL	480
481	FGTSGQKTKI	ISNRGENSCK	ATGQVCHALC	SPEGCWGPEP	RDCVSCRNVS	RGRECVDKCN	540
541	LLEGE PREFV	ENSECIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTC PAVGM	600
600	GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCP TNG	PKIPSIATGM	VGALLLLL LVV	660
661	ALGIGLFMRR	RHIVRKRTL R	RL LQERELVE	PLTPSGEAPN	QALLRILKET	EFKKIKVLGS	720
721	GAFGTVYKGL	WIPEGEKVKI	PVAIKELREA	TSPKANKEIL	DEAYVMASVD	NPHVCRL LGI	780
781	CLTSTVQLIT	QLMPFGCLLD	YVREHKDNIG	SQYLLNWCVQ	IAKGMNYLED	RRLVHRDLAA	840
841	RNVLVKTPQH	VKITDFGLAK	LLGAE EKEYH	AEGGKVPIKW	MALESILHRI	YTHQSDVWSY	900
901	GVTVWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT	IDVYMIMVKC	WMIDADSRPK	960
961	FRELIIIEFSK	MARDPQRYLV	IQGDERMHL P	SPTDSNFYRA	LMDEEDMDDV	VDADEYLIPO	1020
1021	QGGFSSPSTS	RTPLLSSLSA	TSNNSTVACI	DRNLQSCPI	KEDSFLQ RYS	SDPTGALTED	1080
1081	SIDDTFLPVP	EYINQSVPKR	PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN	1140
1141	TVQPTCVNST	FDSPA HWAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS	TAENAEYLRV	1200
1201	APQSSEFIGA						1260

blue: EGF-R sequence expressed in fusion protein **Red:** variant in fusion protein

¹NCBI/Protein accession number NP_005219.2