

Certificate of Analysis

EGF-R L858R

epidermal growth factor receptor

Recombinant Human Active Protein Kinase

HGNC Symbol: EGFR

Synonyms: ERBB, ERBB1

Product No.: 0724-0000-1

Lot: 002

Description: Human EGF-R, C-terminal fragment, amino acids H₆₇₂-A₁₂₁₀ (as in NCBI/Protein entry NP_005219.2), L858R mutant, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: EGF-R L858R Lot 002, was confirmed as EGF-R by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 89,214 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: 0.241 µg/µl

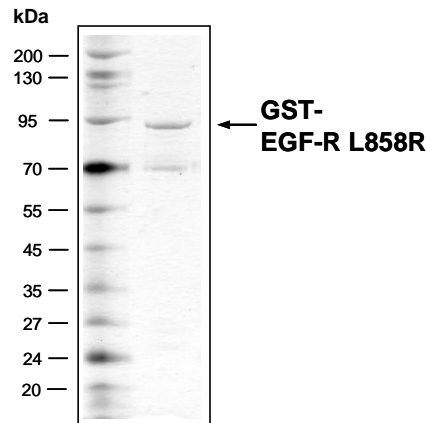
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 48 pmol/µg × min

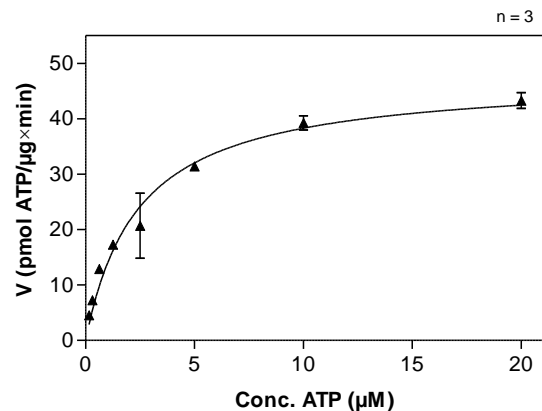
ATP-K_M: 2.4 µM

EGF-R L858R Lot 002: Coomassie stain



2.0 µg GST-EGF-R L858R

EGF-R L858R Lot 002: 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 µg/ml
 - Kinase: 1 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: EGF-R L858R Lot 002

was also successfully tested by ProQinase 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

EGF-R L858R

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GST-EGF-R L858R 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	RDS LEVLFGG	240
241	PLAMGHIVRK	RTLRLQLER	ELVEPLTPSG	EAPNQALLRI	LKETEFKKIK	VLGSGAFGTV	300
301	YKGLWIPEGE	KVKIPVAIKE	LREATSPKAN	KEILDEAYVM	ASVDNPHVCR	LLGICLTSTV	360
361	QLITQLMPFG	CLLDYVREHK	DNIGSQYLLN	WCVQIAKGMN	YLEDRLVHR	DLAARNVLVK	420
421	TPQHVKITDF	GRAKLLGAE	KEYHAEGGKV	PIKWMALESI	LHRIYTHQSD	VWSYGVTVWE	480
481	LMTFGSKPYD	GIPASEISSI	LEKGERLPQP	PICTIDVYMI	MVKCWMIDAD	SRPKFRELI	540
541	EFSKMARDPQ	RYLVIQGDER	MHLPSPTDSN	FYRALMDEED	MDDVVDADAY	LIPQGGFFSS	600
600	PSTSRTPLLS	SLSATSNNST	VACIDRNLQ	SCPIKEDSFL	QRYSSDPTGA	LTEDSIDDTF	660
661	LPVPEYINQS	VPKRPAQSVQ	NPVYHNQPLN	PAPSRDPHYQ	DPHSTAVGNP	EYLNVTQOPTC	720
721	VNSTFDSPAH	WAQKGSQIS	LDNPDYQQDF	FPKEAKPNGI	FKGSTAENAE	YLRVAPQSSE	780
781	FIGA						840

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: EGF-R fragment **boxed**: L858R mutation

EGF-R wt ¹ Amino Acid Sequence							
1	MRPSGTAGAA	LLALLAALCP	ASRALEEKV	CQGTSNKLTQ	LGTTFEDHFLS	LQRMFNCEV	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	TGPRESDECLV	CRKFRDEATC	KDTCPLMLY	NPTTYQMDVN	PEGKYSFGAT	CVKKCPRNYV	300
301	VDHSGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	INATNIKHFK	360
361	NCTSISGDLH	ILPVAFRGDS	FTHTPPLDPQ	ELDILKTVKE	ITGFLLIQAW	PENRTDLHAF	420
421	ENLEIIRGRT	KQHGGQFSLAV	VSLNITSLGL	RSLEISDGD	VIIISGNKNC	YANTINWKKL	480
481	FGTSGQKTKI	ISNRGENSCK	ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCN	540
541	LLEGEPREFV	ENSECIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
600	GENNTLVWKY	ADAGHVCHLC	HPNCTYGCCTG	PGLEGCPNTG	PKIPSIATGM	VGALLLLLVV	660
661	ALGIGLFMRR	RHIVRKRTL	RLQLERELVE	PLTPSGEAPN	QALLRILKMT	EFKKIKVLGS	720
721	GAFGTVYKGL	WIPEGEKVKI	PVAIKELREA	TSPKANKEIL	DEAYVMASVD	NPHVCRLGI	780
781	CLTSTVQLIT	QLMPFGCLLD	YVREHKDNIG	SQYLLNWCVQ	IAKGMNYLED	RRLVHRDLAA	840
841	RNVLVKTPQH	VKITDFGLAK	LLGAEKEYEH	AEGGKVPKW	MALESILHRI	YTHQSDVWSY	900
901	GVTWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT	IDVYMIMVKC	WMIDADSRPK	960
961	FRELIIEFSK	MARDPQRYLV	IQGDERMHL	SPTDSNFYRA	LMDEEDMDDV	VDADEYLIPQ	1020
1021	QGGFFSPSTS	RTPLLSLSA	TSNNSTVACI	DRNGLQSCPI	KEDSFLQYRS	SDPTGALTED	1080
1081	SIDDTFLPVP	EYINQSVPKR	PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN	1140
1141	TVQPTCVNST	FDSPAHWAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS	TAENAEYLRV	1200
1201	APQSSEFIGA						1260

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

¹NCBI/Protein accession number NP_005219.2