

ProQinase™ EGF-R d746-750 T₇₉₀M C₇₉₇S L₈₅₈R

epidermal growth factor receptor

Recombinant Human Active Protein Kinase

HGNC Symbol: EGFR

Synonyms: ERBB, ERBB1

Product No.: 1712-0000-1

Lot: 001

Description: Human EGF-R, C-terminal fragment, amino acids M₆₇₂-A₁₂₁₀ (as in [NCBI/Protein](#) entry NP_005219.2) with a deletion of amino acids E₇₄₆-A₇₅₀ and point mutations T₇₉₀M, C₇₉₇S, L₈₅₈R. N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: EGF-R d746-750 T₇₉₀M C₇₉₇S L₈₅₈R Lot 004, was confirmed as EGF-R by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 88,628 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: 0.328 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

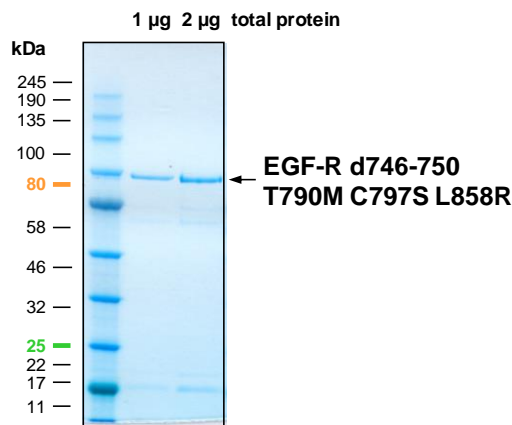
Biochemical Parameters:

Specific kinase activity (P_i transfer): 13 pmol/µg × min
ATP-K_M: 1.1 µM

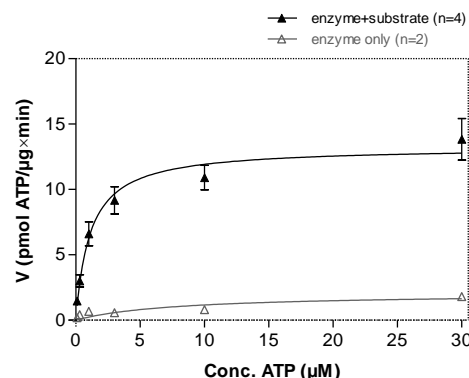
Additional assay technology:

EGF-R d746-750 T₇₉₀M C₇₉₇S L₈₅₈R Lot 004 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 d746-750 T₇₉₀M C₇₉₇S L₈₅₈R Lot 004: Coomassie stain



EGF-R d746-750 T₇₉₀M C₇₉₇S L₈₅₈R Lot 004: Determination of V_{max} and K_M value for ATP



- 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(E/Y)_{4:1} 5 µg/ml
Kinase: 1 µg/ml
- Filter binding assay
MSFC membrane (Millipore)

ProQinase™ EGF-R d746-750 T₇₉₀M C₇₉₇S L₈₅₈R

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GST- EGF-R d746-750 T ₇₉₀ M C ₇₉₇ S L ₈₅₈ R Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGQ	240
241	PLAMGHIVRK	RTLRLQLQER	ELVEPLTPSG	EAPNQALLRI	LKETEFKKIK	VLGSGAFGTQ	300
301	YKGLWIPEGE	KVKIPVAIKT	SPKANKEILD	EAYVMASVDN	PHVCRLLGIC	LTSTVQLIMQ	360
361	LMPFGSLLDY	VREHKDNIGS	QYLLNWCVQI	AKGMNYLEDR	RLVHRDLAAR	NVLVKTPQHV	420
421	KITDFGRLAK	LGAEKEYHA	EGGKVPKIKW	ALESILHRIY	THQSDVWSYG	VTVWELMTFG	480
481	SKPYDGIPAS	EISSILEKGE	RLPQPPICTI	DVYMIMVKCW	MIDADSRPKF	RELIIEFSKM	540
541	ARDPQRYLVI	QGDERMHLPS	PTDSNFYRAL	MDEEDMDDVV	DADEYLIPQ	GFFSSPSTS	600
601	TPLSSLSAT	SNNSTVACID	RNGLQSCPI	EDSFLQRYSS	DPTGALTED	IDDTFLPVE	660
661	YINQSVPKRP	AGSVQNPVYH	HNQPLNPAPS	KPHYQDPHST	AVGNPEYLN	VQPTCVNST	720
721	DSPAHWQKQ	SHQISLDNDP	YQQDFFPKEA	DPNGIFKGST	AENAELYRVA	PQSSEFIGA	780

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: EGF-R fragment boxed: variation from RefSeq

Kinase wt ¹ Amino Acid Sequence							
1	MRPSGTAGAA	LLALLAALCP	ASRALEEKV	CQGTSNKLTQ	LGTTFEDHFLS	LQRMFNANCEV	60
61	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL	IANTVERIP	LENLQIIRGN	MYYENSYALA	120
121	VLSNYDANKT	GLKELPMRNL	QEILHGAVRF	SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	180
181	QNHGSCQKC	DPSCPNGSCW	GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	240
241	TGPRESCLV	CRKFRDEATC	KDTCPLMLY	NPTTYQMDVN	PEGKYSFGAT	CVKKCPRNYV	300
301	VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	INATNIKHPK	360
361	NCTSISGDLH	ILPVAFRGDS	FTHTPPLDQ	ELDILKTVKE	ITGFLLIQAW	PENRTDLHAF	420
421	ENLEIIRGRT	KQHGQFSLAV	VSLNITSLGL	RSLKEISDGD	VIISGNKNLC	YANTINWKKL	480
481	FGTSGQKTKI	ISNRGENSCK	ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCN	540
541	LLEGEPRFV	ENSECIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
600	GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCPTNG	PKIPSIATGM	VGALLLLLVV	660
661	ALGIGLFMRR	RHIVRKRTL	RLQERELVE	PLTPSGEAPN	QALLRILKET	EFKKIKVLGS	720
721	GAFGTVYKGL	WIPEGEKVKI	PVAIKELREA	TSPKANKEIL	DEAYVMASVD	NPHVCRLGI	780
781	CLTSTVQLIT	QLMPFGCLLD	YVREHKDNIG	SQYLLNWCVQ	IAKGMNYLED	RRLVHRDLAA	840
841	RNLVKTPQH	VKITDFGLAK	LLGAEKEYH	AEGGKVPKIKW	MALESILHRI	YTHQSDVWSY	900
901	GVTWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT	IDVYMIMVKC	WMIDADSRPK	960
961	FRELIIEFSK	MARDPQRYLV	IQGDERMHL	SPTDSNFYRA	LMDEEDMDDV	VDAEYLIPQ	1020
1021	QGFFSSPSTS	RTPLSSLSA	TSNNSTVACI	DRNGLQSCPI	KEDSFLQRY	SDPTGALTED	1080
1081	SIDDTFLPVP	EYINQSVPKR	PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN	1140
1141	TVQPTCVNST	FDSPAHWAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS	TAENAELYRVA	1200
1201	APQSSEFIGA						1260

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

¹NCBI/Protein accession number NP_005219.2