

ProQinase™ EGF-R d746-750

epidermal growth factor receptor

Recombinant Human Active Protein Kinase

HGNC Symbol: EGFR

Synonyms: ERBB, ERBB1

Product No.: 1037-0000-1

Lot: 004

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, N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

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

Theoretical MW_{Fusion Protein}: 88,572 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

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: 0.496 µg/µl

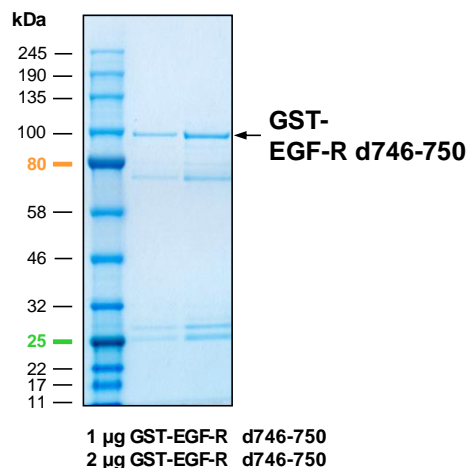
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

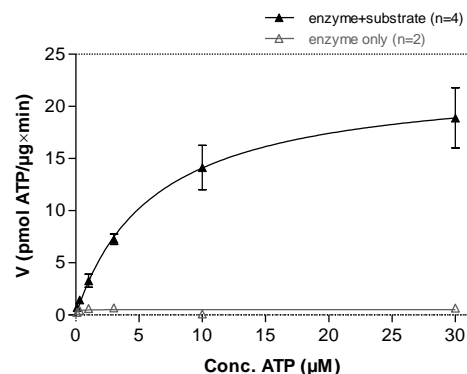
Specific kinase activity (P_i transfer): 23 pmol/µg × min

ATP-K_M: 6.1 µM

EGF-R d746-750 Lot 004: Coomassie stain



EGF-R d746-750 Lot 004: 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 d746-750 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

ProQinase™ EGF-R d746-750

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GST-EGF-R d746-750 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGQ	240
241	PLAMGHIVRK	RTLRLQLQER	ELVEPLTPSG	EAPNQALLRI	LKETEFKKIK	VLGSGAFGTV	300
301	YKGLWIPEGE	KVKIPVAIKT	SPKANKEILD	EAYVMASVDN	PHVCRLLGIC	LTSTVQLITQ	360
361	LMPFGCLLDY	VREHKDNIGS	QYLLNWCVQI	AKGMNYLEDR	RLVHRDLAAR	NVLVKTPQHV	420
421	KITDFGLAKL	LGAEKEYHA	EGGKVPKWM	ALESILHRIY	THQSDVWSYG	VTWELMTFG	480
481	SKPYDGIPAS	EISSILEKGE	RLPQPPICTI	DVYMIMVKCW	MIDADSRPKF	RELIIEFSKM	540
541	ARDPQRYLVI	QGDERMHLPS	PTDSNFYRAL	MDEEDMDDVV	DADEYLIPQ	GFFSSPSTS	600
600	TPLSSLSAT	SNNSTVACID	RNGLQSCPI	EDSFLQRYSS	DPTGALTEDS	IDDTFLPVPE	660
661	YINQSVPKRP	AGSVQNPVYH	NQPLNPAPSR	KPHYQDPHST	AVGNPEYLN	VQPTCVNSTF	720
721	DSPAHWQKQ	GSHQISLDNDP	YQQDFFPKEA	DPNGIFKGST	AENAAYLRVA	PQSSEFIGA	780

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: EGF-R fragment boxed: position of the d746-750 deletion

EGF-R wt ¹ Amino Acid Sequence							
1	MRPSGTAGAA	LLALLAALCP	ASRALEEKV	CQGTSNKLTQ	LGT FEDHFLS	LQRMFN NCEV	60
61	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL	I ALNTVERIP	LENLQIIRGN	MYYENSYALA	120
121	VLSNYDANKT	GLKELPMRNL	QEILHGAVRF	SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	180
181	QNH LGSCQKC	DPSCPNGSCW	GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	240
241	TGPRES DCLV	CRKFRDEATC	KDTCPPMLLY	NPTTYQMDVN	PEGKYSFGAT	CVKKCPRNYV	300
301	VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	INATNIKHF K	360
361	NCTSISGDLH	ILPVAFRGDS	FTHTPPLDPO	ELDILKTVKE	ITGFLLIQAW	PENRTDLHAF	420
421	ENLEIIRGRT	KQH GQFSLAV	VSLNITSLGL	RSLKEISDGD	VIISGNKNLC	YANTINWKKL	480
481	FGTSGQKTKI	ISNRGENSCK	ATGQVCHALC	SPEGCWGPEP	RDCVSCRNVS	RGRECVDKCN	540
541	LLEGEPPREFV	ENSECIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
600	GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLGECPTNG	PKIPSIATGM	VGALLLLL LV	660
661	ALGIGLFMRR	RHIVRKRTL R	RLLQERELVE	PLTPSGEAPN	QALLRILKET	EFKKIKVLGS	720
721	GAFGTVYKGL	WIPEGEKVKI	PVAIKELREA	TSPKANKEIL	DEAYVMASVD	NPHVCRL LGI	780
781	CLTSTVQLIT	QLMPFGCLLD	YVREHKDNIG	SQYLLNWCVQ	IAKGMNYLED	RRLVHRDLAA	840
841	RNVLVKTPQH	VKITDFGLAK	LLGAEKEYEH	AEGGKVPKWK	MALESILHRI	YTHQSDVWSY	900
901	GVTWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT	IDVYMIMVKC	WMIDADSRPK	960
961	FRELIIEFSK	MARDPQRYLV	IQGDERMHL P	SPTDSNFYRA	LMDEEDMDDV	VDADEYLIPQ	1020
1021	QGFFSSPSTS	RTPLSSLSA	TSNNSTVACI	DRNGLQSCPI	KEDSFLQRY S	SDPTGALTED	1080
1081	SIDDTFLPVP	EYINQSVPKR	PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN	1140
1141	TVQPTCVNST	FDSPAHWAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS	TAENAAYLRV	1200
1201	APQSSEFIGA						1260

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

¹NCBI/Protein accession number NP_005219.2