Certificate of Analysis

EGF-R d747-749/A750P

epidermal growth factor receptor

Recombinant Human Active Protein Kinase

Synonyms: ERBB, ERBB1

Product No.: 1038-0000-1

Lot: 001

Description: Human EGF-R, C-terminal fragment, amino acids H_{672}-A_{1210} (as in GenBank entry NM_005228.3), amino acids 747-749 deleted, A750P mutant, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: EGF-R d747-749/A750P Lot 001, was confirmed as EGF-R by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 88,799 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

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

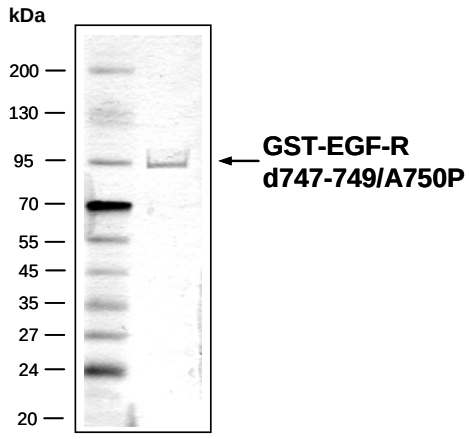
Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 0.150 µg/µl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

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: $Poly(Glu:Tyr)_{4:1}$ (Sigma P-0275), 5.0 µg / ml
  EGF-R d747-749/A750P: 1.0 µg / ml
- Filter binding assay
  MSFC membrane (Millipore)

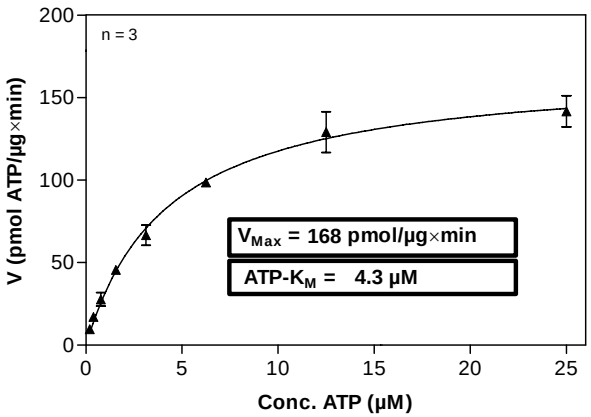
Specific activity: 168 pmol/µg×min

EGF-R d747-749/A750P Lot 001: Coomassie stain



1.0 µg GST-EGF-R d747-749/A750P

EGF-R d747-749/A750P Lot 001: Determination of V_{max} and K_m value for ATP



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EGF-R d747-749/A750P 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 VLGSGAFGTV 300
 301 YKGLWIPEGE KVKIPVAIKE LPTSPKANKEI LDEAYVMASV DNPHVCRLLG ICLTSTVQLI 360
 361 TQLMPFGCLL DYVREHKDNI GSQYLLNWCV QIAKGMNYLE DRRLVHRDLA ARNVLVKTPQ 420
 421 HVKITDFGLA KLLGAEEKEY HAEGGKVPIK WMALESILHR IYTHQSDVWS YGVTVWELMT 480
 481 FGSKPYDGIP ASEISSILEK GERLPQPPIC TIDVYMIMVK CWMIDADSRP KFRELIIEFS 540
 541 KMARDPQRYL VIQGDERMHL PSPTDSNFYR ALMDEEDMDD VVDADEYLIP QQGFFSSPST 600
 601 SRTPLLSSLS ATSNNSTVAC IDRNGLQSCP IKEDSFLQRY SSDPTGALTE DSIDDTFLPV 660
 661 PEYINQSVPK RPAGSVQNPV YHNQPLNPAP SRDPHYQDPH STAVGNPEYL NTVQPTCVNS 720
 721 TFDSPAHWAQ KGSHQISLDN PDYQQDFFPK EAKPNGIFKG STAENAEYLR VAPQSSEFIG 780
 781 A                                                                  840
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1-218: GST **Red: HIS6-tag** **Green: 3C** **blue:EGF-R fragment** **underlined: aa flanking the deletion** **red: point mutation**

EGF-R wt ORF (taken from GenBank entry NM_ 005228.3)

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   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
 601 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: fragment of EGF-R expressed in fusionprotein **RED**: 747-749 deletion A750 mutation site