Certificate of Analysis

REACTION BIOLOGY

ProQinase™ PIK3CB E633K/PIK3R1

phosphoinositide-3-kinase, catalytic, beta polypeptide/
phosphoinositide-3-kinase, regulatory subunit 1 (alpha)

Recombinant Human Active Lipid Kinase

HGNC Symbol: PIK3CB

Synonyms PIK3CB: P110BETA, PI3K, PI3KBETA, PI3K-beta, PIK3C1
Synonyms PIK3R1: GRB1, p85, p85-ALPHA, PtdIns-3-kinase regulatory subunit p85-alpha

Lipid Kinase Family: PI3K Class I
(according to: Phylogenomics of phosphoinositide lipid kinases: perspectives on the evolution of second messenger signaling and drug discovery: James R Brown & Kurt R Auger; BMC Evolutionary Biology 11, 4-14 (2011))

Product No.: 1531-1165-1

Lot: 003

Description: Human PIK3CB, full length, amino acids M_1-S_{1070} (as in NCBI/Protein entry NP_006210.1) with a E633K mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site and PIK3R1 full length, amino acids M_1-R_{724} (as in NCBI/Protein entry NP_852664.1), N-terminal fused to a MYC-tag, expressed in Sf9 insect cells

Product identity: PIK3CB E633K/PIK3R1 Lot 003, was confirmed as PIK3CB/PIK3R1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{GST\text{-}PIK3CB\ E633K}$: 151,266 Da
Theoretical MW_{PIK3R1}: 85,371 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

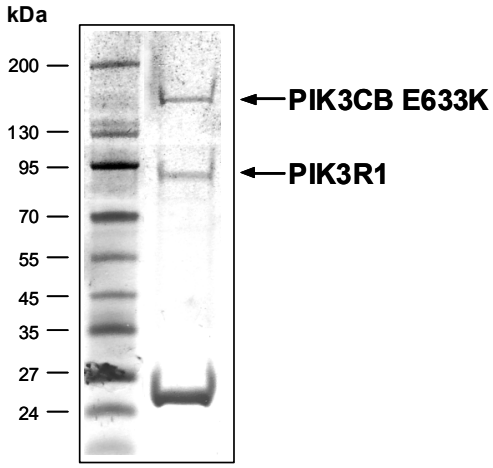
Storage buffer: 50 mM HEPES pH 7.5,
100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!
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: 0.116 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

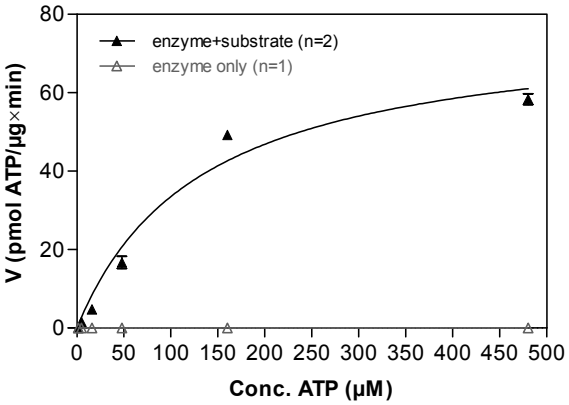
Biochemical Parameters:
Specific kinase activity (P_i transfer): 78 pmol/µg×min
ATP-K_M: 132 µM

PIK3CB E633K/PIK3R1 Lot 003: Coomassie stain



2 µg PIK3CB E633K/PIK3R1

PIK3CB E633K/PIK3R1 Lot 003: Determination of V_{max} and K_M value for ATP ADP-Glo™ Kinase Assay / Promega



Determination of K_M value & Specific activity:
• Assay conditions:
60 mM HEPES-NaOH, pH 7.5
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg / ml $PEG_{20.000}$
ATP (variable)
Substrate: PIP2: 50 µM / PS: 950 µM
PIP2: 08:0 PI(4,5)P2 (1,2-Dioctanoyl-sn-Glycero-3-(Phosphoinositol-4,5-Bisphosphate)
PS: 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-L-Serine])
PIK3CB E633K/PIK3R1: 4.0 µg / ml

For further information on ADP-Glo™ kinase activity detection please visit Promega .com

Recombinant Proteins

REACTION BIOLOGY

ProQinase™ PIK3CB E633K/PIK3R1

Product No.: 1531-1165-1

Recombinant Proteins

PIK3CB E633K Recombinant Fusion Protein Amino Acid Sequence

```
   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 PLAMVMCFSF IMPPAMADIL DIWAVDSQIA SDGSIPVDFL LPTGIYIQLE VPREATISYI 300
 301 KQMLWKQVHN YPMFNLLMDI DSYMFACVNQ TAVYEELEDE TRRLCDVRPF LPVLKLVTRS 360
 361 CDPGEKLDSK IGVLIGKGLH EFDSLKDPEV NEFRRKMRKF SEEKILSLVG LSWMDWLKQT 420
 421 YPPEHEPSIP ENLEDKLYGG KLIVAVHFEN CQDVFSFQVS PNMNPIKVNE LAIQKRLTIH 480
 481 GKEDEVSPYD YVLQVSGRVE YVFGDHPLIQ FQYIRNCVMN RALPHFILVE CCKIKKMYEQ 540
 541 EMIAIEAAIN RNSSNLPLPL PPKKTRIISH VWENNNPFQI VLVKGNKLNT EETVKVHVRA 600
 601 GLFHGTELLC KTIVSSEVSG KNDHIWNEPL EFDINICDLP RMARLCFAVY AVLDKVKTKK 660
 661 STKTINPSKY QTIRKAGKVH YPVAWVNTMV FDFKGQLRTG DIILHSWSSF PDELEEMLNP 720
 721 MGTVQTNPYT ENATALHVKF PENKKQPYYY PPFDKIIEKA AEIASSDSAN VSSRGGKKFL 780
 781 PVLKEILDRD PLSQLCENEM DLIWTLRQDC REIFPQSLPK LLLSIKWNKL EDVAQLQALL 840
 841 QIWPKLPPRE ALELLDFNYP DQYVREYAVG CLRQMSDKEL SQYLLQLVQV LKYEPFLDCA 900
 901 LSRFLLERAL GNRRIGQFLF WHLRSEVHIP AVSVQFGVIL EAYCRGSVGH MKVLSKQVEA 960
 961 LNKLKTLNSL IKLNAVKLNR AKGKEAMHTC LKQSAYREAL SDLQSPLNPC VILSELYVEK 1020
1021 CKYMDSKMKP LWLVYNNKVF GEDSVGVIFK NGDDLRQDML TLQMLRLMDL LWKEAGLDLR 1080
1081 MLPYGCLATG DRSGLIEVVS TSETIADIQL NSSNVAAAAA FNKDALLNWL KEYNSGDDLD 1140
1141 RAIEEFTLSC AGYCVASYVL GIGDRHSDNI MVKKTGQLFH IDFGHILGNF KSKFGIKRER 1200
1201 VPFILTYDFI HVIQQGKTGN TEKFGRFRQC CEDAYLILRR HGNLFITLFA LMLTAGLPEL 1260
1261 TSVKDIQYLK DSLALGKSEE EALKQFKQKF DEALRESWTT KVNWMAHTVR KDYRS      1320
```

1-218: GST **Red: HIS6-tag** **Green:** 3C cleavage site **blue**:PIK3CB **boxed**:E633K

PIK3CB wt[1] amino acid sequence

```
   1 MCFSFIMPPA MADILDIWAV DSQIASDGSI PVDFLLPTGI YIQLEVPREA TISYIKQMLW  60
  61 KQVHNYPMFN LLMDIDSYMF ACVNQTAVYE ELEDETRRLC DVRPFLPVLK LVTRSCDPGE 120
 121 KLDSKIGVLI GKGLHEFDSL KDPEVNEFRR KMRKFSEEKI LSLVGLSWMD WLKQTYPPEH 180
 181 EPSIPENLED KLYGGKLIVA VHFENCQDVF SFQVSPNMNP IKVNELAIQK RLTIHGKEDE 240
 241 VSPYDYVLQV SGRVEYVFGD HPLIQFQYIR NCVMNRALPH FILVECCKIK KMYEQEMIAI 300
 301 EAAINRNSSN LPLPLPPKKT RIISHVWENN NPFQIVLVKG NKLNTEETVK VHVRAGLFHG 360
 361 TELLCKTIVS SEVSGKNDHI WNEPLEFDIN ICDLPRMARL CFAVYAVLDK VKTKKSTKTI 420
 421 NPSKYQTIRK AGKVHYPVAW VNTMVFDFKG QLRTGDIILH SWSSFPDELE EMLNPMGTVQ 480
 481 TNPYTENATA LHVKFPENKK QPYYYPPFDK IIEKAAEIAS SDSANVSSRG GKKFLPVLKE 540
 541 ILDRDPLSQL CENEMDLIWT LRQDCREIFP QSLPKLLLSI KWNKLEDVAQ LQALLQIWPK 600
 601 LPPREALELL DFNYPDQYVR EYAVGCLRQM SDEELSQYLL QLVQVLKYEP FLDCALSRFL 660
 661 LERALGNRRI GQFLFWHLRS EVHIPAVSVQ FGVILEAYCR GSVGHMKVLS KQVEALNKLK 720
 721 TLNSLIKLNA VKLNRAKGKE AMHTCLKQSA YREALSDLQS PLNPCVILSE LYVEKCKYMD 780
 781 SKMKPLWLVY NNKVFGEDSV GVIFKNGDDL RQDMLTLQML RLMDLLWKEA GLDLRMLPYG 840
 841 CLATGDRSGL IEVVSTSETI ADIQLNSSNV AAAAAFNKDA LLNWLKEYNS GDDLDRAIEE 900
 901 FTLSCAGYCV ASYVLGIGDR HSDNIMVKKT GQLFHIDFGH ILGNFKSKFG IKRERVPFIL 960
 961 TYDFIHVIQQ GKTGNTEKFG RFRQCCEDAY LILRRHGNLF ITLFALMLTA GLPELTSVKD 1020
1021 IQYLKDSLAL GKSEEEALKQ FKQKFDEALR ESWTTKVNWM AHTVRKDYRS      1080
```

blue: PIK3CB sequence expressed in fusion protein **Red**: variant in fusion protein

[1]NCBI/Protein accession number NP_006210.1

PIK3R1 Recombinant Fusion Protein Amino Acid Sequence

```
  1  MEEQKLISEE DLPMVMSAEG YQYRALYDYK KEREEDIDLH LGDILTVNKG SLVALGFSDG  60
 61  QEARPEEIGW LNGYNETTGE RGDFPGTYVE YIGRKKISPP TPKPRPPRPL PVAPGSSKTE 120
121  ADVEQQALTL PDLAEQFAPP DIAPPLLIKL VEAIEKKGLE CSTLYRTQSS SNLAELRQLL 180
181  DCDTPSVDLE MIDVHVLADA FKRYLLDLPN PVIPAAVYSE MISLAPEVQS SEEYIQLLKK 240
241  LIRSPSIPHQ YWLTLQYLLK HFFKLSQTSS KNLLNARVLS EIFSPMLFRF SAASSDNTEN 300
301  LIKVIEILIS TEWNERQPAP ALPPKPPKPT TVANNGMNNN MSLQDAEWYW GDISREEVNE 360
361  KLRDTADGTF LVRDASTKMH GDYTLTLRKG GNNKLIKIFH RDGKYGFSDP LTFSSVVELI 420
421  NHYRNESLAQ YNPKLDVKLL YPVSKYQQDQ VVKEDNIEAV GKKLHKYNTQ FQEKSREYDR 480
481  LYEEYTRTSQ EIQMKRTAIE AFNETIKIFE EQCQTQERYS KEYIEKFKRE GNEKEIQRIM 540
541  HNYDKLKSRI SEIIDSRRRL EEDLKKQAAE YREIDKRMNS IKPDLIQLRK TRDQYLMWLT 600
601  QKGVRQKKLN EWLGNENTED QYSLVEDDED LPHHDEKTWN VGSSNRNKAE NLLRGKRDGT 660
661  FLVRESSKQG CYACSVVVDG EVKHCVINKT ATGYGFAEPY NLYSSLKELV LHYQHTSLVQ 720
721  HNDSLNVTLA YPVYAQQRR                                             780
```

Red: MYC-tag **blue: PIK3R1** K: E451K variation

PIK3R1 wt[2] amino acid sequence

```
  1  MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNGYN  60
 61  ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPDLAE 120
121  QFAPPDIAPP LLIKLVEAIE KKGLECSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH 180
181  VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEYI QLLKKLIRSP SIPHQYWLTL 240
241  QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE 300
301  RQPAPALPPK PPKPTTVANN GMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA 360
361  STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFSS VVELINHYRN ESLAQYNPKL 420
421  DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEKS REYDRLYEEY TRTSQEIQMK 480
481  RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID 540
541  SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN 600
601  ENTEDQYSLV EDDEDLPHHD EKTWNVGSSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS 660
661  VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNDSL NVTLAYPVYA 720
721  QQRR                                                             780
```

blue: PIK3R1 sequence expressed in fusion protein **Red**: variant in fusion protein

[2]NCBI/Protein accession number NP_852664.1
E451K: SNP variation see NCBI/dbSNP:rs17852841

Recombinant Proteins