Certificate of Analysis

REACTION BIOLOGY

ProQinase™ PIK3CB/PIK3R1

phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta

Recombinant Human Active Lipid Kinase

HGNC Symbol: PIK3CB

Synonyms: P110BETA, PI3K, PI3KBETA, PI3K-beta, PIK3C1
Synonyms PIK3R1: GRB1, p85, 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 discover: James R Brown & Kurt R Auger; BMC Evolutionary Biology 11, 4-14 (2011))

Product No.: 1168-1165-1

Lot: 011

Description: Human PIK3CB, full length, amino acids M_1-S_{1070} (as in NCBI/Protein entry NP_006210.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, and PIK3R1, full length, amino acids M1-R724 (as in NCBI/Protein entry NP_852664.1), N-terminal fused to a MYC-tag sequence, co-expressed in Sf9 insect cells

Product identity: PIK3CB/PIK3R1 Lot 011, was confirmed as Kinase by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{PIK3CB}: 151,267 Da
Theoretical MW_{PIK3R1}: 85,371 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

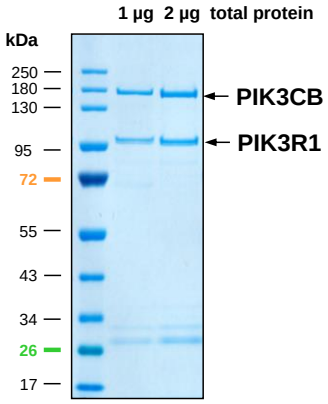
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

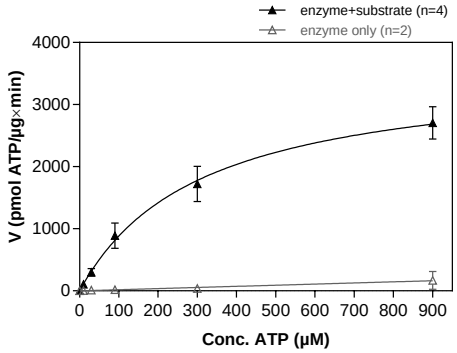
Protein concentration: 0.363 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 3604 pmol/µg x min
ATP-K_M: 308 µM

PIK3CB/PIK3R1 Lot 011:
Coomassie stain



PIK3CB/PIK3R1 Lot 011:
Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  1 mM EGTA
  100 mM NaCl
  0,03% CHAPS
  2 mM DTT
  ATP (variable)
  1% (v/v) DMSO
  Substrate: Example#1 0.0 µg/ml
  Kinase: 1.0 µg/ml
- Assay technology: ADP-Glo (Promega)

Recombinant Proteins

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Sequence information

GST-PIK3CB 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
 600 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 CLRQMSDEEL 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

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 recombinant protein

[1]NCBI/Protein accession number NP_006210.1

Recombinant Proteins

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MYC-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 **boxed**: variation from RefSeq

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 recombinant protein **Red**: variant in recombinant protein

[2]NCBI/Protein accession number NP_852661.1
A100B: SNP variation see NCBI/dbSNP ID: rs17852841
HGNC identifier PIK3R1: PIK3R1

Recombinant Proteins

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