Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ PIK3CA/PIK3R1

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

Recombinant Human Active Lipid Kinase

HGNC Symbol: PIK3CA

Synonyms PIK3CA: p110-alpha, PI3K, PI3K-alpha
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 discovery: James R Brown & Kurt R Auger; BMC Evolutionary Biology 11, 4-14 (2011))

Product No.: 1161-1165-1

Lot: 003

Description: Human PIK3CA, full length, amino acids M_1-N_{1068} (as in NCBI/Protein entry NP_006209.2), N-terminal HIS_6 fusion protein with a Thrombin/TEV 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, coexpressed in Sf9 insect cells

Product identity: PIK3CA/PIK3R1-SV1 Lot 003, was confirmed as PIK3CA/PIK3R1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{PIK3CA} : 130,237 Da
Theoretical MW_{PIK3R1} : 85,371 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: This kinase was not activated by special procedures

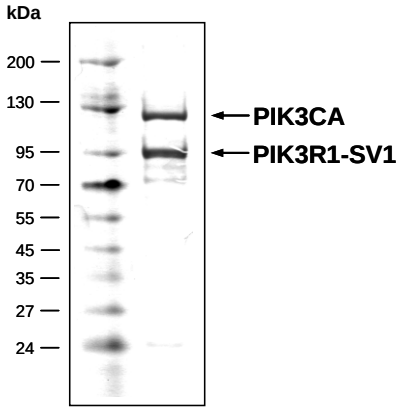
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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

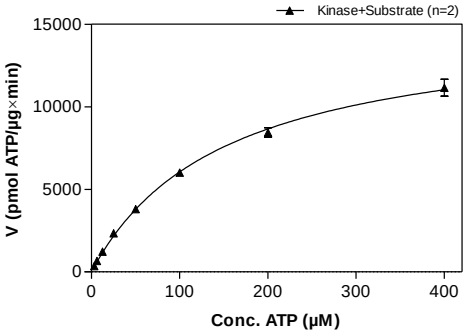
Biochemical Parameters:
Specific kinase activity (P_i transfer): 15189 pmol/µg × min
ATP-K_M: 151 µM

PIK3CA/PIK3R1 Lot 003: Coomassie stain



4 µg PIK3CA/PIK3R1-SV1

PIK3CA/PIK3R1 Lot 003: Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  50 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: 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])
  PIK3CA/PIK3R1: 1 µg/ml
- Assay technology:
  ADP-Glo (Promega)

ProQinase™ PIK3CA/PIK3R1

Product No.: 1161-1165-1

HIS-PIK3CA Recombinant Fusion Protein Amino Acid Sequence

1	MSPIDPMGHH HHHHGRRRAS VAAGILVPRG SPGLDGIYAR TENLYFQGAM GARGRMPPRP	60
61	SSGELWGIHL MPPRILVECL LPNGMIVTLE CLREATLITI KHELFKEARK YPLHQLLQDE	120
121	SSYIFVSVTQ EAEREEFFDE TRRLCDLRLF QPFLKVIEPV GNREEKILNR EIGFAIGMPV	180
181	CEFDMVKDPE VQDFRRNILN VCKEAVDLRD LNSPHSRAMY VYPPNVESSP ELPKHIYNKL	240
241	DKGQIIVVIW VIVSPNNDKQ KYTLKINHDC VPEQVIAEAI RKKTRSMLLS SEQLKLCVLE	300
301	YQGKYILKVC GCDEYFLEKY PLSQYKYIRS CIMLGRMPNL MLMAKESLYS QLPMDCFTMP	360
361	SYSRRISTAT PYMNGETSTK SLWVINSALR IKILCATYVN VNIRDIDKIY VRTGIYHGGE	420
421	PLCDNVNTQR VPCSNPRWNE WLNYDIYIPD LPRAARLCLS ICSVKGRKGA KEEHCPLAWG	480
481	NINLFDYTDT LVSGKMALNL WPVPHGLEDL LNPIGVTGSN PNKETPCLEL EFDWFSSVVK	540
541	FPDMSVIEEH ANWSVSREAG FSYSHAGLSN RLARDNELRE NDKEQLKAIS TRDPLSEITE	600
601	QEKDFLWSHR HYCVTIPEIL PKLLLSVKWN SRDEVAQMYC LVKDWPPIKP EQAMELLDCN	660
661	YPDPMVRGFA VRCLEKYLTD DKLSQYLIQL VQVLKYEQYL DNLLVRFLLK KALTNQRIGH	720
721	FFFWHLKSEM HNKTVSQRFG LLLESYCRAC GMYLKHLNRQ VEAMEKLINL TDILKQEKKD	780
781	ETQKVQMKFL VEQMRRPDFM DALQGFLSPL NPAHQLGNLR LEECRIMSSA KRPLWLNWEN	840
841	PDIMSELLFQ NNEIIFKNGD DLRQDMLTLQ IIRIMENIWQ NQGLDLRMLP YGCLSIGDCV	900
901	GLIEVVRNSH TIMQIQCKGG LKGALQFNSH TLHQWLKDKN KGEIYDAAID LFTRSCAGYC	960
961	VATFILGIGD RHNSNIMVKD DGQLFHIDFG HFLDHKKKKF GYKRERVPFV LTQDFLIVIS	1020
1021	KGAQECTKTR EFERFQEMCY KAYLAIRQHA NLFINLFSMM LGSGMPELQS FDDIAYIRKT	1080
1081	LALDKTEQEA LEYFMKQMND AHHGGWTTKM DWIFHTIKQH ALN	1140

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **Green**: TEV cleavage site **blue**: PIK3CA

PIK3CA wt[1] Amino Acid Sequence

1	MPPRPSSGEL WGIHLMPPRI LVECLLPNGM IVTLECLREA TLITIKHELF KEARKYPLHQ	60
61	LLQDESSYIF VSVTQEAERE EFFDETRRLC DLRLFQPFLK VIEPVGNREE KILNREIGFA	120
121	IGMPVCEFDM VKDPEVQDFR RNILNVCKEA VDLRDLNSPH SRAMYVYPPN VESSPELPKH	180
181	IYNKLDKGQI IVVIWVIVSP NNDKQKYTLK INHDCVPEQV IAEAIRKKTR SMLLSSEQLK	240
241	LCVLEYQGKY ILKVCGCDEY FLEKYPLSQY KYIRSCIMLG RMPNLMLMAK ESLYSQLPMD	300
301	CFTMPSYSRR ISTATPYMNG ETSTKSLWVI NSALRIKILC ATYVNVNIRD IDKIYVRTGI	360
361	YHGGEPLCDN VNTQRVPCSN PRWNEWLNYD IYIPDLPRAA RLCLSICSVK GRKGAKEEHC	420
421	PLAWGNINLF DYTDTLVSGK MALNLWPVPH GLEDLLNPIG VTGSNPNKET PCLELEFDWF	480
481	SSVVKFPDMS VIEEHANWSV SREAGFSYSH AGLSNRLARD NELRENDKEQ LKAISTRDPL	540
541	SEITEQEKDF LWSHRHYCVT IPEILPKLLL SVKWNSRDEV AQMYCLVKDW PPIKPEQAME	600
601	LLDCNYPDPM VRGFAVRCLE KYLTDDKLSQ YLIQLVQVLK YEQYLDNLLV RFLLKKALTN	660
661	QRIGHFFFWH LKSEMHNKTV SQRFGLLLES YCRACGMYLK HLNRQVEAME KLINLTDILK	720
721	QEKKDETQKV QMKFLVEQMR RPDFMDALQG FLSPLNPAHQ LGNLRLEECR IMSSAKRPLW	780
781	LNWENPDIMS ELLFQNNEII FKNGDDLRQD MLTLQIIRIM ENIWQNQGLD LRMLPYGCLS	840
841	IGDCVGLIEV VRNSHTIMQI QCKGGLKGAL QFNSHTLHQW LKDKNKGEIY DAAIDLFTRS	900
901	CAGYCVATFI LGIGDRHNSN IMVKDDGQLF HIDFGHFLDH KKKKFGYKRE RVPFVLTQDF	960
961	LIVISKGAQE CTKTREFERF QEMCYKAYLA IRQHANLFIN LFSMMLGSGM PELQSFDDIA	1020
1021	YIRKTLALDK TEQEALEYFM KQMNDAHHGG WTTKMDWIFH TIKQHALN	1080

blue: PIK3CA sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_006209.2

Recombinant Proteins

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MYC-PIK3R1 Recombinant Fusion Protein Amino Acid Sequence

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   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
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1-218: GST **Red**: MYC-tag **blue**: PIK3R1 **boxed**: variation from RefSeq

PIK3R1 wt[2] Amino Acid Sequence

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   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
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blue: PIK3R1 sequence expressed in recombinant protein

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