

ProQinase™ PIK3CB D1067A/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 discover: James R Brown & Kurt R Auger; BMC Evolutionary Biology 11, 4-14 (2011))

Product No.: 1584-1165-1

Lot: 001

Description: Human PIK3CB, full length, amino acids M₁-S₁₀₇₀ (as in [NCBI/Protein](#) entry NP_006210.1) with a D1067A mutation, N-terminal GST-HIS₆ 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, expressed in Sf9 insect cells

Product identity: PIK3CB D1067A/PIK3R1 Lot001, has been verified by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{PIK3CB}: 151221 Da
Theoretical MW_{PIK3R1}: 85371 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!

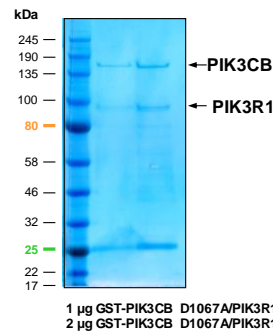
Protein concentration: 0.134 µg/µl

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

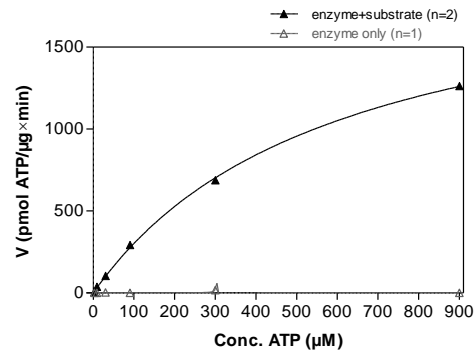
Biochemical Parameters:

Specific kinase activity (P_i transfer): 2089 pmol/µg x min
ATP-K_M: 592 µM

PIK3CB D1067A/PIK3R1 Lot 001: Coomassie stain



PIK3CB D1067A/PIK3R1 Lot 001: Determination of V_{max} and K_M value for ATP



• Assay conditions:

- 60 mM HEPES-NaOH, pH 7.5
- 3 mM MnCl₂
- 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 D1067A/PIK3R1: 4.0 µg/ml

- Assay technology: ADP-Glo (Promega)

Sequence information

GST-PIK3CB D1067A Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RDSLEVLFGG	240
241	PLAMVMCFSF	IMPPAMADIL	DIWAVDSQIA	SDGSIPVDFL	LPTGIYIQLE	VPREATISYI	300
301	KQMLWKQVHN	YPMFNLLMDI	DSYMFACVNO	TAVYEELEDE	TRRLCDVRPF	LPVLKLVTRS	360
361	CDPGEKLD SK	IGVLIGKGLH	EFDSLKDPEV	NEFRKRMRKF	SEEKILSLVG	LSWMDWLKQT	420
421	YPPEHEPSIP	ENLEDKLYGG	KLIVAVHFEN	CQDVFSFQVS	PNMNPIKVNE	LAIQKRLTIH	480
481	GKEDEVSPYD	YVLQVSGRVE	YVFGDHPLIQ	FQYIRNCVMN	RALPHFILVE	CCKIKKMYEQ	540
541	EMIAIEAAIN	RNSSNLPLPL	PPKKTRIISH	VWENNNPFQI	VLVKGKNLNT	EETVKVHVRA	600
601	GLFHGTELLC	KTIVSSEVSG	KNDHIWNEPL	EFDINICDLP	RMARLCFAVY	AVLDKVKTKK	660
661	STKTINPSKY	QTIRKAGKVH	YPVAVVNTMV	FDFKGQLRTG	DIILHSWSSF	PDELEEMLNP	720
721	MGTVQTNPYT	ENATALHVKF	PENKKQPYYY	PPFDKIIIEKA	AEIASSDSAN	VSSRGGKFFL	780
781	QVKEILD RD	PLVQLCENEM	DLIWTLRQDC	REIFPQSLPK	LLLSIKWNKL	EDVAQLQALL	840
841	QIWPKLPPRE	ALELLDFNYP	DQYVREYAVG	CLRQMSDEEL	SQYLLQLVQV	LKYEPFLDCA	900
901	LSRFL LERAL	GNRRIGQFLF	WHLRSEVHIP	AVSVQFGVIL	EAYCRGVS GH	MKVLSKQVEA	960
961	LNKLKTLNSL	IKLNAVKLN R	AKGKEAMHTC	LKQSA YREAL	SDLQSPLNPC	VILSELYVEK	1020
1021	CKYMDSKMKP	LWL VYNNKVF	GEDSVGVIFK	NGDDL RQDML	TLQMLRLMDL	LWKEAGL DLR	1080
1081	MLPYGCLATG	DRSGLIEVVS	TSETIADIQL	NSSNVA AAAA	FNKDALLNWL	KEYNSGDDLD	1140
1141	RAIEEFTLSC	AGYCVASYVL	GIGDRHSDNI	MVKKYGQLFH	IDFGHILQVF	KSKFKGKRRER	1200
1201	VPFILT YDFI	HVIQGGKTGN	TEKFRFRQNC	CEDAYLILRR	HGNLFITLFA	LMLTAGLPEL	1260
1261	TSVKDIQY LK	DSLALGKSEE	EALKQFKQKF	DEALRESWTT	KVNWMAHTVR	KAYRS	1320

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue:PIK3CB boxed: D1067

PIK3CB wt ¹ Amino Acid Sequence							
1	MCFSFIMPPA	MADILDIWAV	DSQIASD GSI	PVDFLLPTGI	YIQLEVPREA	TISYIKQMLW	60
61	KQVHNYP MFN	LLMDIDS YMF	ACVNQTAVYE	ELEDETRRLC	DVRPFLPVLK	LVTRSCDPGE	120
121	KLDSKIGVLI	GKGLHEFDSL	KDPEVNEFR R	KMRKFSEEKI	LSLVGLSWMD	WLKQTY PPEH	180
181	EPSIPENLED	KLYGGKLI VA	VHFENCQDVF	SFQVSPNMNP	IKVNELAIQK	RLTIHGKED E	240
241	VSPYDYVLQV	SGRVEYVFGD	HPLIQFQYIR	NCVMNRALPH	FILVECKKIK	KMYEQEMIAI	300
301	EAAINRNSSN	LPLPLPPKKT	RIISHVWENN	NPFQIVLVKG	NKLNTEETVK	VHVRA GLFHG	360
361	TELLCKTIVS	SEVSGKNDHI	WNEPLEFDIN	ICDLPRMARI	CFAVYAVLDK	VKTKKSTKTI	420
421	NPSKYQTIRK	AGKVHY PVAW	VNTMVDFDFK	QLRTGDIILH	SWSSF PDELE	EMLNPMGTVQ	480
481	TNPYTENATA	LHVKFPENKK	QPYYYPFDK	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	LYVECKYMD	780
781	SKMKPLWL VY	NNKVFGE DSV	GVIFKNGDDL	RQDMLTLQML	RLMDLLWKEA	GLDLRMLPYG	840
841	CLATGDRSGL	IEVSTSETI	ADIQLNSSNV	AAAAAFNKDA	LLNWLKEYNS	GDDLDRATEE	900
901	FTLSCAGYCV	ASYVLGIGDR	HSDNIMVKKT	GQLFHIDFGH	ILGNFKSKFG	IKRERV PFIL	960
961	TYDFIHVIQQ	GKTGNT EFKG	RFRQCCEDAY	LILRRHG NLF	ITLFALMLTA	GLPELTSVKD	1020
1021	IQYLKDSLAL	GKSEEEALQ	FKQKFDEALR	ESWTTKVNWM	AHTVRKDYRS		1080

blue: PIK3CB sequence expressed in recombinant protein Red: variant in recombinant protein

¹NCBI/Protein accession number NP_006210.1

PIK3R1 Recombinant Fusion Protein Amino Acid Sequence								
1	MEEQKLISEE	DL	PMVMSAEG	YQYRALYDYK	KEREEDIDLH	LGDILTVMKG	SLVALGFSDG	60
61	QEARPEEIGW	LNGYNETTGE	RGDFPGTYVE	YIGRKKISPP	TPKPRPPRPL	PVAPGSSKTE		120
121	ADVEQQALTL	PDLAEQFAPP	DIAPPLLIK	VEAIEKKGLE	CSTLYRTQSS	SNLAELRQLL		180
181	DCDTPSVGLE	MIDVHVLADA	FKRYLLDLPN	PVIPAAYVSE	MISLAPEVQS	SEEYIQLLKK		240
241	LIRSPSIPHQ	YWLTLOYLK	HFFKLSQTSS	KNLLNARVLS	EIFSPMLFRF	SAASSDNTE		300
301	LIKVIEILIS	TEWNERQPAP	ALPPKPPKPT	TVANNGMNN	MSLQDAEWY	GDISREEVNE		360
361	KLRDTADGTF	LVRDASTKMH	GDYTLTLRKG	GNNKLIKIFH	RDGKYGFSDP	LTFSSVVELI		420
421	NHYRNESLAQ	YNPKLDVKLL	YPVSKYQQDQ	VVKEDNIEAV	GKKLHKYNTQ	FQEKRSREYDR		480
481	LYEEYTRTSQ	EIQMKRTAIE	AFNETIKIFE	EQCQTQERY	KEYIEKFKRE	GNEKEIQRIM		540
541	HNYDKLKSRI	SEIIDSRRRL	EEDLKKQAAE	YREIDKRMNS	IKPDLIQLRK	TRDQYLMWLT		600
601	QKGVRRQKLN	EWLGNENTED	QYSLVEDDED	LPHHDEKTNW	VGSSNRNKAE	NLLRGKRDGT		660
661	FLVRESSKQG	CYACSVVDG	EVKHCVINKT	ATGYGFAEPY	NLYSSLKELV	LHYQHTSLVQ		720
721	HNDLSLNVTLA	YPVYAQQRR						780

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

PIK3R1 wt ² Amino Acid Sequence							
1	MSAEGYQYRA	LYDYKKERE	DIDLHLGDIL	TVNKGSLVAL	GFSGDQEARP	EEIGWLNGYN	60
61	ETTGERGDFP	GTIVEYIGRK	KISPPTPKPR	PPRPLPVAPG	SSKTEADVEQ	QALTLPLDLAE	120
121	QFAPPDIAPP	LLIKLVEAIE	KKGLECSTLY	RTQSSSNLAE	LRQLLCDTTP	SVDLEMIDVH	180
181	VLADAFKRYL	LDLPNPVIPA	AVYSEMISLA	PEVQSSEEI	QLLKKLIRSP	SIPHQYWLTL	240
241	QYLLKHFFKL	SQTSSKNLLN	ARVLSEIFSP	MLFRFSAASS	DNTENLIKVI	EILISTEWNE	300
301	RQPAPALPPK	PPKPTTVANN	GMNNMSLQD	AEWYWGDISR	EEVNEKLRDT	ADGTFLVRDA	360
361	STKMHGDYTL	TLRKGNNKL	IKIFHRDGKY	GFSDDLTFSS	VVELINHYRN	ESLAQYNPKL	420
421	DVKLLYPVSK	YQQDQVVKED	NIEAVGKKLH	EYNTQFQEK	REYDRLYEEY	TRTSQEIQMK	480
481	RTAIEAFNET	IKIFEEQCQT	QERYSKEYIE	KFKREGNEKE	IQRIMHNYDK	LKSRISEIID	540
541	SRRLEEDLK	KQAAEYREID	KRMNSIKPDL	IQLRKTDRDY	LMWLTQKGV	QKKNLNEWLGN	600
601	ENTEDQYSLV	EDDEDLPHHD	EKTWNVGSSN	RNKAENLLRG	KRDGTFVRE	SSKQGCYACS	660
661	VVDGEVKHC	VINKTATGYG	FAEPYNLYSS	LKELVLHYQH	TSLVQHNDL	NVTLAYPVYA	720
721	QQRR						780

blue: PIK3R1 sequence expressed in recombinant protein Red: variant in recombinant protein

²NCBI/Protein accession number NP_852664.1

E451K: SNP variation see NCBI/dbSNP ID: rs17852841