

ProQinase™ PIK3CB D1067Y/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.: 1583-1165-1

Lot: 001

Description: Human PIK3CB, full length, amino acids M₁-S₁₀₇₀ (as in NCBI/Protein entry NP_006210.1) with a D1067Y 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 D1067Y/PIK3R1 Lot 001, was confirmed as PIK3CB/PIK3R1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-PIK3CB D1067Y}: 151,315 Da

Theoretical MW_{PIK3R1}: 85,371 Da

Expression host: Sf9 insect 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

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: 0.211 µg/µl

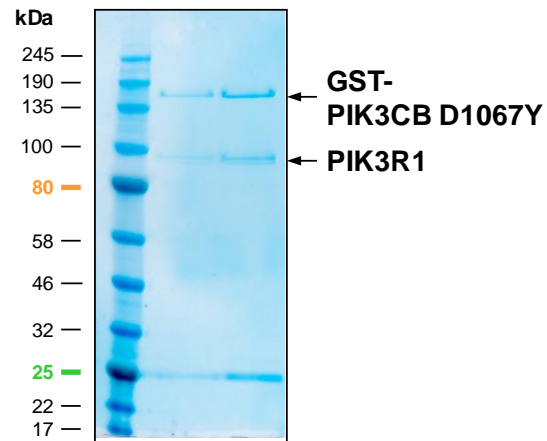
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 2604 pmol/µg×min

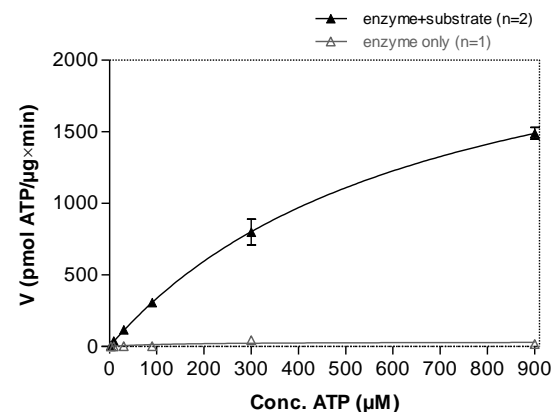
ATP-K_M: 674 µM

**PIK3CB D1067Y/PIK3R1 Lot 001:
Coomassie stain**



1 µg GST-PIK3CB D1067Y/PIK3R1
2 µg GST-PIK3CB D1067Y/PIK3R1

**PIK3CB D1067Y/PIK3R1 Lot 001:
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 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 D1067Y/PIK3R1: 4 µg/ml

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

ProQinase™ PIK3CB D1067Y/PIK3R1

Product No.: 1583-1165-1

GST-PIK3CB D1067Y Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGQ	240
241	PLAMVMCFSSF	IMPAMADIL	DIWA VDSQIA	SDGSIPVDFL	LPTGIYIQLE	VPREATISYI	300
301	KQMLWKQVHN	YPMFNLLMDI	DSYMFACVNO	TAVYEELEDE	TRRLCDVRPF	LPVLKLVTRS	360
361	CDPGEKLD SK	IGVLIGKGLH	EFD SLKDPEV	NEFRRKMRKF	SEEKILSLVG	LSWMDWLKQT	420
421	YPPEHEPSIP	ENLEDKLYGG	KLIVAVHFEN	CQDVFSFQVS	PNMNPIK VNE	LAIQKRLTIH	480
481	GKEDEVSPYD	YVLQVSGRVE	YVFGDHPLIQ	FQYIRNCVMN	RALPHFILVE	CCKIKKMYEQ	540
541	EMIAIEAAIN	RNSSNLPLPL	PPKKTRII SH	VWENNNPFQI	VLVKG NKLNT	EETVKVHVRA	600
601	GLFHGTELLC	KTIVSSEVSG	KNDHIWNEPL	EFDINICDLP	RMARLCFAVY	AVLDKVKTKK	660
661	STKTINPSKY	QTIRKAGKVH	YPVAWVNTMV	FDKQGLRTG	DIILHSWSSF	PDELEEMLNP	720
721	MGTVQTNPYT	ENATALHV KF	PENKKQPYYY	PPFDKII EKA	AEIASSDSAN	VSSRGGKKFL	780
781	PVLKEILDRD	PLSQLCENEM	DLIWTLRQDC	REIFPQSLPK	LLLSIKWNKL	EDVAQLQALL	840
841	QIWPKLPPRE	ALELLDFNYP	DQYVREYAVG	CLRQMSDEEL	SOYLLQLVQV	LKYEPFLDCA	900
901	LSRFL LERAL	GNRRIGQFLF	WHLRSEVHIP	AVSVQFGVIL	EAYCRGSVGH	MKVL SKQVEA	960
961	LNKLKT LNSL	IKLNAVKLNR	AKGKEAMHTC	LKQSAYREAL	SDLQSP LNPC	VILSELYVEK	1020
1021	CKYMDSKMKP	LWLVYNNKVF	GEDSVGVIFK	NGDDL RQDML	TLQMLRLMDL	LWKEAGL DLR	1080
1081	MLPYGCLATG	DRSGLIEVVS	TSETIADIQL	NSSNVAAAAA	FNKDALLNWL	KEYNSGDDLD	1140
1141	RAIEEFTLSC	AGYCVASYVL	GIGDRHSDNI	MVKKTGQLFH	IDFGHILGNF	KSKFGIKRER	1200
1201	VPFILTYDFI	HVIQQGKTGN	TEKFGFRQC	CEDAYLILRR	HGNLFITLFA	LMLTAGLPEL	1260
1261	TSVKDIQYLK	DSLALGKSEE	EALKQFKQKF	DEALRESWTT	KVNWMAHTVR	KYRS	1320

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue:PIK3CB boxed: D1067Y

PIK3CB wt ¹ amino acid sequence							
1	MCFSFIMPPA	MADILDIWAV	DSQIASD GSI	PVDFLLPTGI	YIQLEVPREA	TISYIKQMLW	60
61	KQVHNYPMFN	LLMDIDSYMF	ACVNQTAVYE	ELEDETRRLC	DVRPFLPVLK	LVTRSCDPGE	120
121	KLDSKIGVLI	GKGLHEFDSL	KDPEVNEFRR	KMRKFSEEKI	LSLVGLSWMD	WLKQTYPPPEH	180
181	EPSIPENLED	KLYGGKLIVA	VHFENCQDVF	SFQVSPNMNP	IKVNELAIQK	RLTIHGKEDE	240
241	VSPYDYVLQV	SGRVEYVFGD	HPLIQFQYIR	NCVMNRALPH	FILVECKKIK	KMYEQEMIAI	300
301	EAAINRNSSN	LPLPLPPKKT	RIISHVWENN	NPFQIVLVKG	NKLNTEETVK	VHVRAGLFHG	360
361	TELLCKTIVS	SEVSGKNDHI	WNEPLEFDIN	ICDLPRMARL	CFAYAVL DK	VKTKKSTKTI	420
421	NPSKYQTIRK	AGKVHYVPAW	VNTMVDFDKG	QLRTGDIILH	SWSSF PDELE	EMLNPMGTVQ	480
481	TNPYTENATA	LHVKFPENKK	QPYYP PFDK	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	LYVEALKCYMD	780
781	SKMKPLWL VY	NNKVFGE DSV	GVI FKN GDDL	RQDMLTLQML	RLMDLLWKEA	GLDLRMLPYG	840
841	CLATGDRSGL	IEVSTSETI	ADIQLNSSNV	AAAAAFNKDA	LLNWLKEYNS	GDDLDR AIEE	900
901	FTLSCAGYCV	ASYVLGIGDR	HSDNIMVKKT	GQLFHIDFGH	ILGNFKSKFG	IKRERV PFIL	960
961	TYDFI HVIQQ	GKTGNT EFKG	RFRQCCEDAY	LILRRHGNLF	ITL FALMLTA	GLPELTSVKD	1020
1021	IQY LKDSLAL	GKSEEEALQ	FKQKFDEALR	ESWTTKV NWM	AHTVRKDYRS		1080

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

¹NCBI/Protein accession number NP_006210.1

Recombinant Proteins

PIK3R1 Recombinant Fusion Protein Amino Acid Sequence

1	MEEQKLISEE	DL	PMVMSAEG	YQYRALYDYK	KEREEDIDLH	LGDILTVNKG	SLVALGFSDG	60
61	QEARPEEIGW	LNGYNETTGE	RGDFPGTYVE	YIGRKKISPP	TPKPRPPRPL	PVAPGSSKTE	120	
121	ADVEQQALTL	PDLAEQFAPP	DIAPPLLIK	VEAIEKKGLE	CSTLYRTQSS	SNLAE LRQLL	180	
181	DCDTPSVDLE	MIDVHVLADA	FKRYLLDLPN	PVIPAAVYSE	MISLAPEVQS	SEEYIQLLKK	240	
241	LIRSPSIPHQ	YWLTLYLLK	HFFKLSQTSS	KNLLNARVLS	EIFSPMLFRF	SAASSDNTEN	300	
301	LIKVIEILIS	TEWNERQPAP	ALPPKPPKPT	TVANNGMNN	MSLQDAEWYW	GDISREEVNE	360	
361	KLRDTADGTF	LVRDASTKMH	GDYTLTLRKG	GNNKLIKIFH	RDGKYGFSDP	LTFSVVELI	420	
421	NHYRNESLAQ	YNPKLDVKLL	YPVSKYQQDQ	VVKEDNIEAV	GKKLHKYNTQ	FQEKREYDR	480	
481	LYEYTRTSQ	EIQMKRTAIE	AFNETIKIFE	EQCQTQERY	KEYIEKFKRE	GNEKEIQRIM	540	
541	HNYDKLKSRI	SEIIDSRRRL	EEDLKKQAAE	YREIDKRMNS	IKPDLIQLRK	TRDQYLMWLT	600	
601	QKGVROKQLN	EWLGNENTED	QYSLVEDDED	LPHHDEKTWN	VGSSNRNKA	NLLRGKRDGT	660	
661	FLVRESSKQG	CYACSVVDG	EVKHCVINKT	ATGYGFAEPY	NLYSSLKELV	LHYQHTSLVQ	720	
721	HNDLNVTLA	YPVYAQQR					780	

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

PIK3R1 wt² amino acid sequence

1	MSAEGYQYRA	LYDYKKEREE	DIDLHLGDIL	TVNKGSLVAL	GFSDGQEARP	EEIGWLNQYN	60
61	ETTGERGDFP	GTVEYIGRK	KISPPTPKPR	PPRPLPVAPG	SSKTEADVEQ	QALTLPLDLAE	120
121	QFAPPDIAPP	LLIKLVEAIE	KKGLECSTLY	RTQSSSNLAE	LRQLLDCTP	SVDLEMIDVH	180
181	VLADAFKRYL	LDLPNPVIPA	AVYSEMISLA	PEVQSSEEYI	QLLKKLIRSP	SIPHQYWLTTL	240
241	QYLLKHFFKL	SQTSSKNLLN	ARVLSEIFSP	MLFRFSAASS	DNTENLIKVI	EILISTEWNE	300
301	RQPAPALPPK	PPKPTTVANN	GMNNMSLQD	AEWYWGDISR	EEVNEKLRDT	ADGTFLVRDA	360
361	STKMHGDYTL	TLRKGNNKL	IKIFHRDGKY	GFSDPLTFSS	VVELINHYRN	ESLAQYNPKL	420
421	DVKLLYPVSK	YQQDQVVKED	NIEAVGKKLH	EYNTQFQEK	REYDRLYEY	TRTSQEIQMK	480
481	RTAIEAFNET	IKIFEEQCQT	QERYKEYIE	KFKREGNEKE	IQRIMHNYDK	LKSRISEIID	540
541	SRRRLEEDLK	KQAAEYREID	KRMNSIKPDL	IQLRKTRDQY	LMWLTQKGV	QKKLNEWLGN	600
601	ENTEDQYSLV	EDDEDLPHHD	EKTWNVGSSN	RNKAENLLRG	KRDGTFLVRE	SSKQGCYACS	660
661	VVDGEVVKHC	VINKTATGYG	FAEPYNLYSS	LKELVLHYQH	TSLVQHNDL	NVTLAYPVYA	720
721	QQR						780

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

²NCBI/Protein accession number NP_852664.1

E451K: SNP variation see NCBI/dbSNP:rs17852841