

# Certificate of Analysis



## PIK3CB D1067V/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.: 1533-1165-1

Lot: 002

**Description:** Human PIK3CB, full length, amino acids M<sub>1</sub>-S<sub>1070</sub> (as in NCBI/Protein entry NP\_006210.1) with a D1067V mutation, N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site and PIK3R1 full length, amino acids M<sub>1</sub>-R<sub>724</sub> (as in NCBI/Protein entry NP\_852664.1), N-terminal fused to a MYC-tag, expressed in Sf9 insect cells

**Product identity:** PIK3CB D1067V/PIK3R1 Lot 002, was confirmed as PIK3CB/PIK3R1 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>GST-PIK3CB D1067V</sub>:** 151,261 Da

**Theoretical MW<sub>PIK3R1</sub>:** 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

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:** 0.184 µg/µl

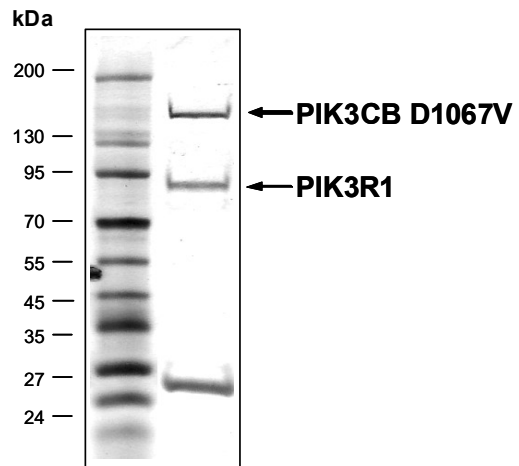
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 813 pmol/µg×min

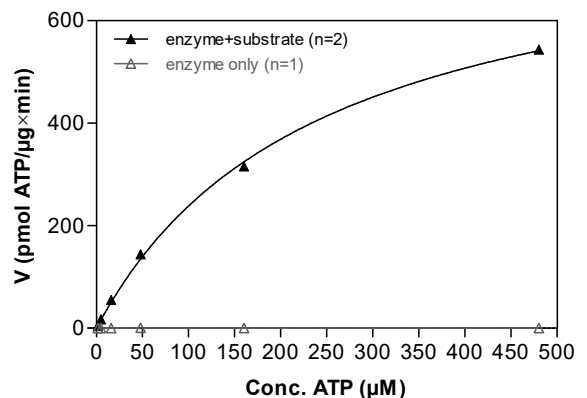
ATP-K<sub>M</sub>: 241 µM

### PIK3CB D1067V/PIK3R1 Lot 002: Coomassie stain



2 µg PIK3CB D1067V/PIK3R1

### PIK3CB D1067V/PIK3R1 Lot 002: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP ADP-Glo™ Kinase Assay / Promega



### Determination of K<sub>M</sub> value & Specific activity:

#### • Assay conditions:

60 mM HEPES-NaOH, pH 7.5

3 mM MnCl<sub>2</sub>

3 µM Na-orthovanadate

1.2 mM DTT

50 µg / ml PEG<sub>20,000</sub>

ATP (variable)

Substrate: PIP<sub>2</sub>: 50 µM / PS: 950 µM

PIP<sub>2</sub>: 08:0 PI(4,5)P<sub>2</sub> (1,2-Dioctanoyl-sn-Glycero-3-(Phosphoinositol-4,5-Bisphosphate))

PS: 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-L-Serine])

PIK3CB D1067V/PIK3R1: 4.0 µg / ml

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



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CoA V8.1 PIK3CB\_D1067V\_PIK3R1\_Lot002\_V2.doc

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## PIK3CB D1067V/PIK3R1

Product No.: 1533-1165-1

PIK3CB D1067V Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHG</b>	RDS <b>LEVLFGQ</b>	240
241	<b>PLAMVMCFSF</b>	<b>IMPPAMADIL</b>	<b>DIWAVDSQIA</b>	<b>SDGSIPVDFL</b>	<b>LPTGIYIQLE</b>	<b>VPREATISYI</b>	300
301	<b>KQMLWKQVHN</b>	<b>YPMFNLLMDI</b>	<b>DSYMFACVNO</b>	<b>TAVYEELEDE</b>	<b>TRRLCDVRPF</b>	<b>LPVLKLVTRS</b>	360
361	<b>CDPGEKLDK</b>	<b>IGVLIGKGLH</b>	<b>EFDSLKDPEV</b>	<b>NEFRRKMRKF</b>	<b>SEEKILSLVG</b>	<b>LSWMDWLKQT</b>	420
421	<b>YPPEHEPSIP</b>	<b>ENLEDKLYGG</b>	<b>KLIVAVHFEN</b>	<b>CQDVFSFQVS</b>	<b>PNMNPIKUNE</b>	<b>LAIQKRLTIH</b>	480
481	<b>GKEDEVSPYD</b>	<b>YVLQVSGRVE</b>	<b>YVFGDHPLIQ</b>	<b>FQYIRNCVMN</b>	<b>RALPHFILVE</b>	<b>CKIKKMYEQ</b>	540
541	<b>EMIAIEAAN</b>	<b>RNSSNLPLPL</b>	<b>PKKTRIIISH</b>	<b>VWENNNPFOI</b>	<b>VLVKGKLNLT</b>	<b>EETVKVHVRA</b>	600
601	<b>GLFHGTTELLC</b>	<b>KTIVSSEVSG</b>	<b>KNDHIWNEPL</b>	<b>EFDINICDLP</b>	<b>RMARLCFAVY</b>	<b>AVLDKVKTKK</b>	660
661	<b>STKTINPSKY</b>	<b>QTIRKAGKVH</b>	<b>YPVAWNTMV</b>	<b>FDKQQLRTG</b>	<b>DIILHSWSSF</b>	<b>PDELEEMLNP</b>	720
721	<b>MGTVQTNPYT</b>	<b>ENATALHVKF</b>	<b>PENKQPYYY</b>	<b>PPFDKIEKA</b>	<b>AEIASSDSAN</b>	<b>VSSRGGKFKL</b>	780
781	<b>PVLKEILDRD</b>	<b>PLSQLCENEM</b>	<b>DLIWTLRQDC</b>	<b>REIFPQSLPK</b>	<b>LLLSIKWNKL</b>	<b>EDVAQLQALL</b>	840
841	<b>QIWPKLPPRE</b>	<b>ALELLDFNYP</b>	<b>DQYVREYAVG</b>	<b>CLRQMSDEEL</b>	<b>SQYLLQLVQV</b>	<b>LKYEPFLDCA</b>	900
901	<b>LSRFLLERAL</b>	<b>GNRRIGQFLF</b>	<b>WHLRSEVHIP</b>	<b>AVSVQFGVIL</b>	<b>EAYCRGSVGH</b>	<b>MKVLKQVEA</b>	960
961	<b>LNKLTLSL</b>	<b>IKLNAVKLN</b>	<b>AKGKEAMHTC</b>	<b>LKQSAAREAL</b>	<b>SDLQSPLNPC</b>	<b>VILSELYVEK</b>	1020
1021	<b>CKYMDSKMKP</b>	<b>LWLVYNNKVF</b>	<b>GEDSVGVIFK</b>	<b>NGDDLQDML</b>	<b>TLQMLRLMDL</b>	<b>LWKEAGDLR</b>	1080
1081	<b>MLPYGCLATG</b>	<b>DRSGLIEVVS</b>	<b>TSETIADIQL</b>	<b>NSSNVAASAA</b>	<b>FNKDALLNWL</b>	<b>KEYNSGDDLD</b>	1140
1141	<b>RAIEEFTLSC</b>	<b>AGYCVASYVL</b>	<b>GIGDRHSDNI</b>	<b>MVKKTGQLFH</b>	<b>IDFGHILGNF</b>	<b>KSKFGIKRER</b>	1200
1201	<b>VPFILTYDFI</b>	<b>HVIQOGKTGN</b>	<b>TEKFRFRQC</b>	<b>CEDAYLILRR</b>	<b>HGNLFITLFA</b>	<b>LMLTAGLPEL</b>	1260
1261	<b>TSVKDIQYLK</b>	<b>DSLALGKSEE</b>	<b>EALKQFKQKF</b>	<b>DEALRESWTT</b>	<b>KVNWMAHTVR</b>	<b>KVYRS</b>	1320

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**:PIK3CB **boxed**: D1067V

PIK3CB wt <sup>1</sup> amino acid sequence							
1	<b>MCFSFIMPPA</b>	<b>MADILDIWAV</b>	<b>DSQIASDGS</b>	<b>PVDFLLPTGI</b>	<b>YIQLEVPREA</b>	<b>TISYIKQMLW</b>	60
61	<b>KQVHNYPMFN</b>	<b>LLMDIDSYMF</b>	<b>ACVNQTAVYE</b>	<b>ELEDETRRLC</b>	<b>DVRPFLPVLK</b>	<b>LVTRSCDPGE</b>	120
121	<b>KLDSKIGVLI</b>	<b>GKGLHEFDSL</b>	<b>KDPEVNEFR</b>	<b>KMRKFSEEKI</b>	<b>LSLVGLSWMD</b>	<b>WLKQTYPPPEH</b>	180
181	<b>EPSIPENLED</b>	<b>KLYGGKLIVA</b>	<b>VHFENCQDVF</b>	<b>SFQVSPNMNP</b>	<b>IKVNELAIQK</b>	<b>RLTIHGKEDE</b>	240
241	<b>VSPYDYVLQV</b>	<b>SGRVEYVFGD</b>	<b>HPLIQFQYIR</b>	<b>NCVMNRALPH</b>	<b>FILVECKIK</b>	<b>KMYEQEMIAI</b>	300
301	<b>EAAINRNSSN</b>	<b>LPLPLPPKKT</b>	<b>RIISHVWENN</b>	<b>NPFQIVLVKG</b>	<b>NKLNTEETVK</b>	<b>VHVRAGLFHG</b>	360
361	<b>TELLCKTIVS</b>	<b>SEVSGKNDHI</b>	<b>WNEPLEFDIN</b>	<b>ICDLPRMARL</b>	<b>CAVAVLVDK</b>	<b>VKTKKSTKTI</b>	420
421	<b>NPSKYQTIRK</b>	<b>AGKVHYPVAW</b>	<b>VNTMVDFDKG</b>	<b>QLRTGDIILH</b>	<b>SWSSFPDELE</b>	<b>EMLNPMGTVQ</b>	480
481	<b>TNPYTENATA</b>	<b>LHVKFPENKK</b>	<b>QPYYPFFDK</b>	<b>IEKAAEIAS</b>	<b>SDSANVSSRG</b>	<b>GKKFLPVLKE</b>	540
541	<b>ILDRDPLSQL</b>	<b>CENEMDLIWT</b>	<b>LRQDCREIFP</b>	<b>QSLPKLLLSI</b>	<b>KWNKLEDVAQ</b>	<b>LQALLQIWPK</b>	600
601	<b>LPPREALELL</b>	<b>DFNYPDQYVR</b>	<b>EYAVGCLRQM</b>	<b>SDEELSQYLL</b>	<b>QLVQVLKYEP</b>	<b>FLDCALSRFL</b>	660
661	<b>LERALGNRRI</b>	<b>GQFLFWHLRS</b>	<b>EVHIPAVSVQ</b>	<b>FGVILEAYCR</b>	<b>GSVGHMKVLS</b>	<b>KQVEALNKLK</b>	720
721	<b>TLNSLIKLNA</b>	<b>VKLNRAKGE</b>	<b>AMHTCLKQSA</b>	<b>YREALSDLQS</b>	<b>PLNPCVILSE</b>	<b>LYVECKYMD</b>	780
781	<b>SKMKPLWLVI</b>	<b>NNKVFGEDEV</b>	<b>GVIKNGDDL</b>	<b>RQDMLTLQML</b>	<b>RLMDLLWKEA</b>	<b>GLDLRMLPYG</b>	840
841	<b>CLATGDRSGL</b>	<b>IEVSTSETI</b>	<b>ADIQLNSSNV</b>	<b>AAAAAFNKDA</b>	<b>LLNWLKEYNS</b>	<b>GDDLDRATIEE</b>	900
901	<b>FTLSCAGYCV</b>	<b>ASYVLGIGDR</b>	<b>HSDNIMVKKT</b>	<b>QGLFHIDFGH</b>	<b>ILGNFKSKFG</b>	<b>IKRERVFFIL</b>	960
961	<b>TYDFIHVIQ</b>	<b>GKTGNTKFG</b>	<b>RFRQCCEAD</b>	<b>LILRRHGNLF</b>	<b>ITLFAALMLTA</b>	<b>GLPELTSVKD</b>	1020
1021	<b>IQYLKDSLAL</b>	<b>GKSEEEALKQ</b>	<b>FKQKFDEALR</b>	<b>ESWTTKVNWM</b>	<b>AHTVRKDYRS</b>		1080

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

<sup>1</sup>NCBI/Protein accession number NP\_006210.1

PIK3R1 Recombinant Fusion Protein Amino Acid Sequence						
1	M <b>EEQKLISEE</b> DLPMVMSAEG YQYRALYDYK KEREEDIDLH LGDILTVNKG SLVALGFSDBG	60				
61	QEARPEEIGW LNGYNETTGE RGDFPGTYVE YIGRKKISPP TPKRPPRPL PVAPGSSKTE	120				
121	ADVEQQALTL PDLAEQFAPP DIAPPLLIK LVEAIEKKGLE CSTLYRTQSS SNLAE LRQLL	180				
181	DCDTPSVDLE MIDVHVLADA FKRYLLDLPN PVIPAAVYSE MISLAPEVQS SEEYIQLLKK	240				
241	LIRSPSIPHQ YWLTQLYLLK HFFKLSQTSS KNLLNARVLS EIFSPMLFRF SAASSDNTEN	300				
301	LIKVIEILIS TEWNERQPAP ALPPKPKPT TVANNGMNN MSLQDAEWYW GDISREEVNE	360				
361	KLRDTADGTF LVRDASTKMH GDYTLTLRKG GNNKLIKIFH RDGKYGFSDP LTFSSVELI	420				
421	NHYRNESLAQ YNPKLDVKLL YPVSKYQQDQ VVKEDNIEAV GKKLH <b>K</b> YNTQ FQEKSRDYDR	480				
481	LYEYTRTSQ EIQMRTAIE AFNETIKIFE EQCQTQERYS KEYIEKFKRE GNEKEIQRIM	540				
541	HNYDKLSRI SEIIDSRRRL EEDLKKQAAE YREIDKRMNS IKPDLIQLRK TRDQYLMWLT	600				
601	QKGVROKKNL EWLGNENTED QYSLVEDDED LPHHDEKTWN VGSSNRNKA E NLLRGKRDGT	660				
661	FLVRESSKQG CYACSVVDG EVKHCVINKT ATGYGFAEPY NLYSSLKELV LHYQHTSLVQ	720				
721	HNSLNVTLA YPVYAQQRR	780				

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

PIK3R1 wt <sup>2</sup> amino acid sequence						
1	MSAEGYQYRA LYDYKKERE DIDLHLGDIL TVNKGSLVAL GFSDBGQEARP EEIGWLNLYN	60				
61	ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDAE	120				
121	QFAPPDIAPP LLIKLV E AIE KKGLE CSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH	180				
181	VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEYI QLLKKLIRSP SIPHQYWLTL	240				
241	QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE	300				
301	RQPAPALPPK PPKPTTVANN GMNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA	360				
361	STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDDLTFSS VVELINHYRN ESLAQYNPKL	420				
421	DVKLLYPVSK YQQDQVVKED NIEAVGKKLH <b>E</b> YNTQFQEKSRDYDRLYEY TRTSQEIQMK	480				
481	RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID	540				
541	SRRLEEDLK QAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVROKKNLNEWLGN	600				
601	ENTEDQYSLV EDDDLPHHD EKTWNVGSSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS	660				
661	VVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNSL NVTLAYPVYA	720				
721	QQRR	780				

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

<sup>2</sup>NCBI/Protein accession number NP\_852664.1  
E451K: SNP variation see NCBI/dbSNP:rs17852841