

ProQinase™ PIK3CD/PIK3R1

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

Recombinant Human Active Lipid Kinase

HGNC Symbol: PIK3CD

Synonyms PIK3CD: p110D, P110DELTA, PI3K, PI3K-delta

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.: 1162-1165-1

Lot: 004

Description: Human PIK3CD, full length, amino acids M₁-Q₁₀₄₄ (as in [NCBI/Protein](#) entry NP_005017.3), 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-terminally fused to a MYC-tag, coexpressed in Sf9 insect cells

Product identity: PIK3CD/PIK3R1 Lot 004, was confirmed as PIK3CD/PIK3R1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{GST-PIK3CD} : 147,998 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: 0.250 µg/µl

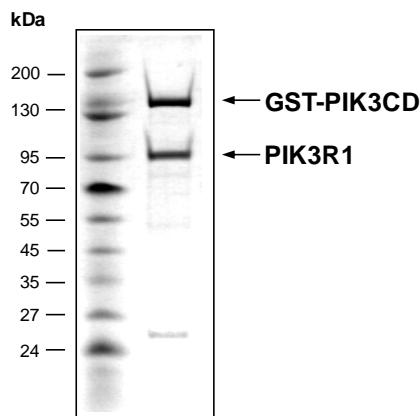
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 14967pmol/µg × min

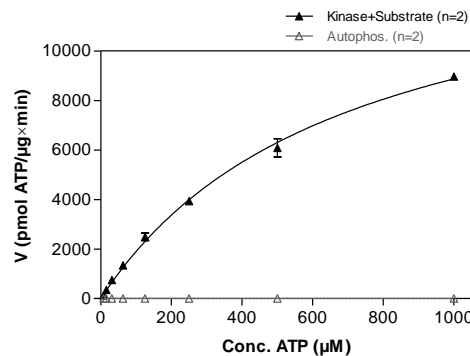
ATP-K_M: 688 µM

**PIK3CD/PIK3R1 Lot 004:
Coomassie stain**



4 µg GST-PIK3CD/PIK3R1

**PIK3CD/PIK3R1 Lot 004:
Determination of V_{max} and K_M value for ATP**



• Assay conditions:

60 mM HEPES-NaOH, pH 7.5

3 mM MgCl₂

3 mM MnCl₂

3 µM Na-orthovanadate

1.2 mM DTT

50 µg/ml PEG_{20,000}

ATP (variable)

Substrate: PIP2/PS (1:9) 150 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)

Kinase: 8.6 nM

• Assay technology: ADP-Glo (Promega)

ProQinase™ PIK3CD/PIK3R1

Product No.: 1162-1165-1

GST-PIK3CD Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQG WQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFCG	240
241	PLAMLMPPGV	DCPMEFWTKE	ENQSVVDFL	LPTGVYLNFP	VSRNANLSTI	KQLLWHRAQY	300
301	EPLFHM LSGP	EAYVFTCINQ	TAEQ QELEDE	QRRLCDVQPF	LPVLR LVARE	GDRVK KLINS	360
361	QISLLIGKGL	HEFDSLCDPE	VNDFRAKMCQ	FCEEEAARRQ	QLGWEAWLQY	SFPLQLEPSA	420
421	QTWGP GTLRL	PNRALLVNK	FEGSEESFTF	QVSTKDVPLA	LMACALRKA	TVFRQPLVEQ	480
481	PEDYTLQVNG	RHEYLYGSYP	LCQFQYICSC	LHSGLTPHLT	MVHSSSILAM	RDEQSNPAPQ	540
541	VQKPRAKPPP	IPAKKPSSVS	LWSLEQPFRI	ELIQGSKVNA	DERMKLVVQA	GLFHGNEMLC	600
600	KTVSSSEVSV	CSEP VVKQRL	EFDINICDLP	RMARLCFALY	AVIEKAKKAR	STKKKSKKAD	660
661	CPIAWANLML	FDYKDLKGTG	ERCLYMWPSV	PDEK GELLNP	TGTVRSNPNT	DSAAALLICL	720
721	PEVAPHPVYV	PALEKILELG	RHSECVHVTE	EEQLQ LREIL	ERRGSGELYE	HEKDLVWKL R	780
781	HEVQEHFPEA	LARLLLVTWK	NKHEDVAQML	YLLCSWPELP	VLSALELLDF	SFPDCHVGSF	840
841	AIKSLRKLTD	DELFOYLLQL	VQVLKYESYL	DCELT KFLLD	RALANR KIGH	FLFWHLRSEM	900
901	HVPSVALRFG	LILEAYCRGS	THHMKVL MKQ	GEALS K LKAL	NDFVKLSSQK	TPKPQTKELM	960
961	HLCMRQEAYL	EALSHLQSP L	DPSTLLAEVC	VEQCTF MDSK	MKPLWIMYSN	EEAGSGG SVG	1020
1021	IIFKNGDDL R	QDMLTLQMIQ	LMDVLWKQEG	LDLRMT PYGC	LPTGDR TGLI	EVVLRSDTIA	1080
1081	NIQLNKS NMA	ATAAFNKDAL	LNWLKSNPGE	EALDRAIEEF	TLSCAGY CVA	TYVLLIGDRH	1140
1141	SDNIMIRESG	QLFHIDFGHF	LGNFKTKFGI	NRERV PFILT	YDFVHV IQQG	KTNNSEKFER	1200
1201	FRGYCERAYT	ILRRHGLLFL	HLFALMRAAG	LP ELSCKSDI	OY LKDSLALG	KTEEEALKHF	1260
1261	RVKFNEALRE	SWKTKVNWLA	HNVSKDN RQ				1320

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: PIK3CD

PIK3CD wt ¹ Amino Acid Sequence							
1	MPPGVDCPME	FWTKEENQSV	VVDFLLPTGV	YLNFPVSRNA	NLSTIKQLLW	HRAQYEPLFH	60
61	MLSGPEAYVF	TCINQTAEQQ	ELEDEQRRLC	DVQPFLPVL R	LVAREGDRVK	KLINSQI SLL	120
121	IGKGLHEFDS	LCDPEVNDFR	AKMCQFCEEA	AARRQQLGWE	AWLQYSFPLQ	LEPSAQTWGP	180
181	GTLRLPNRAL	LVNVKFEGSE	ESFTFQVSTK	DVPLALMACA	LRKKATVFRQ	PLVEQPEDYT	240
241	LQVNGRHEYL	YGSYPLCQFQ	YIC SCLHSGL	TPHLTMVHSS	SILAMRDEQS	NPAPQVQKPR	300
301	AKPPP IPAK	PSSVSLWSLE	QPFRIELIQG	SKVNADERMK	LVVQAGLFHG	NEMLCKTVSS	360
361	SEVSVCS EPG	WKQRLEFDIN	ICDLPRMARI	CFALYAVIEK	AKKARSTKKK	SKKADCP IAW	420
421	ANLMLFDYKD	QLKTGERCLY	MWPSVPDEKG	ELLNPTGTVR	SNPNTDSAAA	LLICLPEVAP	480
481	HPVYYPALEK	I LELGRHSEC	VHVTEEEQLQ	LREILERRGS	GELYEHEKDL	VWKL RHEVQE	540
541	HFPEALARLL	LVTKWNKHED	VAQMLYLLCS	WPELPVLSAL	ELLD FSFPDC	HVGSFAIKSL	600
600	RKLTDDDEL FQ	YLLQLVQVLK	YESYLDCELT	KFLLDRALAN	RKIGHFLFWH	LRSEMHVPSV	660
661	ALRFG LILEA	YCRGSTHMK	VLMKQGEALS	KLKALNDFVK	LSSQKTPK PQ	TKELMHLCMR	720
721	QEAYLEALSH	LQSP LDPSTL	LAEVCVEQCT	FMSKMKPLW	IMYSNEEAGS	GGSVGIIFKN	780
781	GDDL RQDMLT	LQMIQLMDVL	WKQEGDLDRM	TPYGC LPTGD	RTGLIEV VLR	SDTIANIQLN	840
841	KSNMAATAAF	NKDALLNW LK	SKNPGEALDR	AIEEFTLSCA	GYCVATYV LG	IGDRHSDNIM	900
901	IRESGQLFHI	DFGHFLGNFK	TKFGINRERV	PFILTYDFVH	VIQQGKTNNS	EKFERFRGYC	960
961	ERAYTILRRH	GLLFLHLFAL	MRAAGLP ELS	CSKDIQYLKD	SLALGKTEEE	ALKHFRVKFN	1020
1021	EALRESWKTK	VNWLAHNVSK	DNRQ				1080

blue: PIK3CD sequence expressed in recombinant protein

¹NCBI/Protein accession number NP_005017.3

MYC-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	SNLAELRQLL		180
181	DCDTPSVdle	MIDVHVLADA	FKRYLLDLPN	PVIPAAYVSE	MISLAPEVQS	SEEIYQLLKK		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	QKQVRRQKLN	EWLGNATED	QYSLVEDDED	LPHHDEKTNW	VGSSNRNKAE	NLLRQKRDGT		660
661	FLVRESSKQG	CYACSVVDG	EVKHCVINKT	ATGYGFAEPY	NLYSSLKELV	LHYQHTSLVQ		720
721	HNDSLNVTLA	YPVYAQQRR						780

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

PIK3R1 wt² Amino Acid Sequence

1	MSAEGYQYRA	LYDYKKEREE	DIDLHLGDIL	TVNKGSLVAL	GFSDGQEARP	EEIGWLNQYN	60
61	ETTGERGDFP	GTyVEYIGRK	KISPPTPKPR	PPRPLPVAPG	SSKTEADVEQ	QALTLPLDLAE	120
121	QFAPPDIAPP	LLIKLVEAIE	KGLEcSTLY	RTQSSSNLAE	LRQLLDcDTP	SVDLEMIDVH	180
181	VLADAFKRYL	LDLpNPVIPA	AVYSEMISLA	PEVQSSEYI	QLLKKLIRSP	SIPHQYWLTL	240
241	QYLLKHFFKL	SQTSSKNLLN	ARVLSEIFSP	MLFRFSAASS	DNTENLIKVI	EILISTEWNE	300
301	RQPAPALPPK	PPKPTTVANN	GMNNMSLQD	AEWYWGDISR	EEVNEKLRTD	ADGTFVLRDA	360
361	STKMHGDYTL	TLRKGGNKl	IKIFHRDGKY	GFSdPLTFSS	VVELINHYRN	ESLAQYNPKL	420
421	DVKLLYPVSK	YQDQVVKED	NIEAVGKKLH	EYNTQFQEKs	REYDRLYEEY	TRTSQEIOMK	480
481	RTAIEAFNET	IKIFEEQCQT	QERYsKEYIE	KFKREGNEKE	IQRIMHNYDK	LKSRISEIID	540
541	SRRLEEDLK	QAAEYREID	KRMNSIKPDL	IQLRKTRDQY	LMWLTQKGVR	QKKLNEWLGN	600
601	ENTEDQYSLV	EDDEDLPHHD	EKTWNVGSSN	RNKAENLLRG	KRDGTFVRE	SSKQGCYACS	660
661	VVDGGEVKHC	VINKTATGYG	FAEPYNLYSS	LKELVLHYQH	TSLVQHNDSL	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