Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ PIK3CG

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

Recombinant Human Active Lipid Kinase

HGNC Symbol: PIK3CG

Synonyms: p120-PI3K, PI3CG, PI3K, PI3K-gamma, PIK3

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.: 1163-0000-1

Lot: 002

Description: Human PIK3CG, full length, amino acids M_1-A_{1102} (as in NCBI/Protein entry NP_002640.2), N-terminal HIS_6 fusion protein with a Thrombin and TEV cleavage site, expressed in Sf9 insect cells

Product identity: PIK3CG Lot 002, was confirmed as PIK3CG by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 132,406 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: This kinase was not activated by special procedures

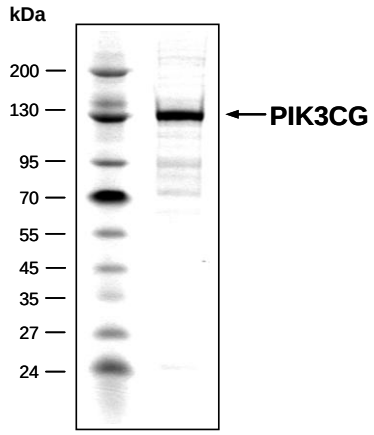
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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

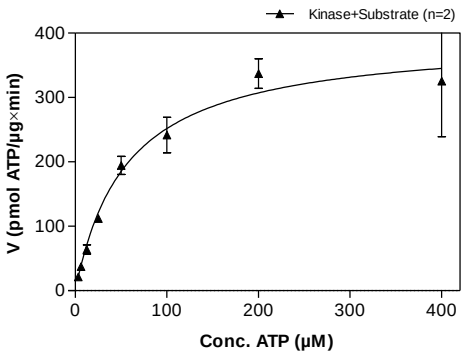
Biochemical Parameters:
Specific kinase activity (P_i transfer): 394 pmol/µg × min
ATP-K_M: 56 µM

PIK3CG Lot 002:
Coomassie stain



2 µg PIK3CG

PIK3CG Lot 002:
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 MgCl2
1 mM EGTA
100 mM NaCl
0,03% CHAPS
2 mM DTT
ATP: variable concentration
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])
PIK3CG: 1 µg/ml
• Assay technology:
ADP-Glo (Promega)

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ProQinase™ PIK3CG

Product No.: 1163-0000-1

HIS-PIK3CG Recombinant Fusion Protein Amino Acid Sequence

1	MSPIDPMGHH HHHHGRRRAS VAAGILVPRG SPGLDGIYAR TENLYFQGAM GARGRMELEN	60
61	YKQPVVLRED NCRRRRRMKP RSAAASLSSM ELIPIEFVLP TSQRKCKSPE TALLHVAGHG	120
121	NVEQMKAQVW LRALETSVAA DFYHRLGPHH FLLLYQKKGQ WYEIYDKYQV VQTLDCLRYW	180
181	KATHRSPGQI HLVQRHPPSE ESQAFQRQLT ALIGYDVTDV SNVHDDELEF TRRGLVTPRM	240
241	AEVASRDPKL YAMHPWVTSK PLPEYLWKKI ANNCIFIVIH RSTTSQTIKV SPDDTPGAIL	300
301	QSFFTKMAKK KSLMDIPESQ SEQDFVLRVC GRDEYLVGET PIKNFQWVRH CLKNGEEIHV	360
361	VLDTPPDPAL DEVRKEEWPL VDDCTGVTGY HEQLTIHGKD HESVFTVSLW DCDRKFRVKI	420
421	RGIDIPVLPR NTDLTVFVEA NIQHGQQVLC QRRTSPKPFT EEVLWNVWLE FSIKIKDLPK	480
481	GALLNLQIYC GKAPALSSKA SAESPSSESK GKVQLLYYVN LLLIDHRFLL RRGEYVLHMW	540
541	QISGKGEDQG SFNADKLTSA TNPDKENSMS ISILLDNYCH PIALPKHQPT PDPEGDRVRA	600
600	EMPNQLRKQL EAIIATDPLN PLTAEDKELL WHFRYESLKH PKAYPKLFSS VKWGQQEIVA	660
661	KTYQLLARRE VWDQSALDVG LTMQLLDCNF SDENVRAIAV QKLESLEDDD VLHYLLQLVQ	720
721	AVKFEPYHDS ALARFLLKRG LRNKRIGHFL FWFLRSEIAQ SRHYQQRFAV ILEAYLRGCG	780
781	TAMLHDFTQQ VQVIEMLQKV TLDIKSLSAE KYDVSSQVIS QLKQKLENLQ NSQLPESFRV	840
841	PYDPGLKAGA LAIEKCKVMA SKKKPLWLEF KCADPTALSN ETIGIIFKHG DDLRQDMLIL	900
901	QILRIMESIW ETESLDLCLL PYGCISTGDK IGMIEIVKDA TTIAKIQQST VGNTGAFKDE	960
961	VLNHWLKEKS PTEEKFQAAV ERFVYSCAGY CVATFVLGIG DRHNDNIMIT ETGNLFHIDF	1020
1021	GHILGNYKSF LGINKERVPF VLTPDFLFVM GTSGKKTSPH FQKFQDICVK AYLALRHHTN	1080
1081	LLIILFSMML MTGMPQLTSK EDIEYIRDAL TVGKNEEDAK KYFLDQIEVC RDKGWTVQFN	1140
1141	WFLHLVLGIK QGEKHSA	1200

Red: HIS6-tag **Pink**: Thrombin cleavage site **Green**: TEV cleavage site **blue**: PIK3CG

PIK3CG wt[1] Amino Acid Sequence

1	MELENYKQPV VLREDNCRRR RRMKPRSAAA SLSSMELIPI EFVLPTSQRK CKSPETALLH	60
61	VAGHGNVEQM KAQVWLRALE TSVAADFYHR LGPHHFLLLY QKKGQWYEIY DKYQVVQTLD	120
121	CLRYWKATHR SPGQIHLVQR HPPSEESQAF QRQLTALIGY DVTDVSNVHD DELEFTRRGL	180
181	VTPRMAEVAS RDPKLYAMHP WVTSKPLPEY LWKKIANNCI FIVIHRSTTS QTIKVSPDDT	240
241	PGAILQSFFT KMAKKKSLMD IPESQSEQDF VLRVCGRDEY LVGETPIKNF QWVRHCLKNG	300
301	EEIHVVLDTP PDPALDEVRK EEWPLVDDCT GVTGYHEQLT IHGKDHESVF TVSLWDCDRK	360
361	FRVKIRGIDI PVLPRNTDLT VFVEANIQHG QQVLCQRRTS PKPFTEEVLW NVWLEFSIKI	420
421	KDLPKGALLN LQIYCGKAPA LSSKASAESP SSESKGKVQL LYYVNLLLID HRFLLRRGEY	480
481	VLHMWQISGK GEDQGSFNAD KLTSATNPDK ENSMSISILL DNYCHPIALP KHQPTPDPEG	540
541	DRVRAEMPNQ LRKQLEAIIA TDPLNPLTAE DKELLWHFRY ESLKHPKAYP KLFSSVKWGQ	600
600	QEIVAKTYQL LARREVWDQS ALDVGLTMQL LDCNFSDENV RAIAVQKLES LEDDDVLHYL	660
661	LQLVQAVKFE PYHDSALARF LLKRGLRNKR IGHFLFWFLR SEIAQSRHYQ QRFAVILEAY	720
721	LRGCGTAMLH DFTQQVQVIE MLQKVTLDIK SLSAEKYDVS SQVISQLKQK LENLQNSQLP	780
781	ESFRVPYDPG LKAGALAIEK CKVMASKKKP LWLEFKCADP TALSNETIGI IFKHGDDLRQ	840
841	DMLILQILRI MESIWETESL DLCLLPYGCI STGDKIGMIE IVKDATTIAK IQQSTVGNTG	900
901	AFKDEVLNHW LKEKSPTEEK FQAAVERFVY SCAGYCVATF VLGIGDRHND NIMITETGNL	960
961	FHIDFGHILG NYKSFLGINK ERVPFVLTPD FLFVMGTSGK KTSPHFQKFQ DICVKAYLAL	1020
1021	RHHTNLLIIL FSMMLMTGMP QLTSKEDIEY IRDALTVGKN EEDAKKYFLD QIEVCRDKGW	1080
1081	TVQFNWFLHL VLGIKQGEKH SA	1140

blue: PIK3CG sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002640.2

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