

Certificate of Analysis



PI4K2A

phosphatidylinositol 4-kinase type 2 alpha

Recombinant Human Active Lipid Kinase

HGNC Symbol: PI4K2A

Synonyms: PI4KII, PIK42A

Lipid Kinase Family: PI4K Class II

(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.: 1202-0000-1

Lot: 001

Description: Human PI4K2A, full length, amino acids M₁-W₄₇₉ (as in NCBI/Protein entry NP_060895.1), N-terminal GST-HIS₆ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: PI4K2A Lot 001, was confirmed as PI4K2A by mass spectroscopy LC-ESI-MS/MS

Activation: This kinase was not activated by special procedures

Theoretical MW_{Fusion Protein}: 82,111 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, 0.1 % Triton X-100, 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: 0.262 µg/µl

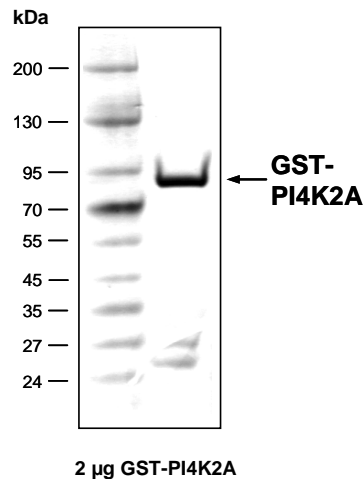
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

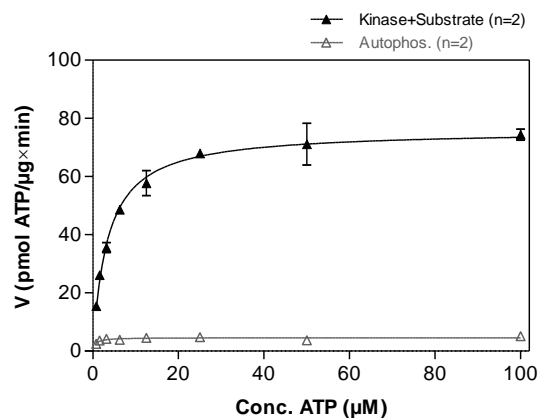
Specific kinase activity (P_i transfer): 76 pmol/µg×min

ATP-K_M: 3.4 µM

PI4K2A Lot 001: Coomassie stain



PI4K2A 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 mixture:

50 mM HEPES-NaOH, pH 7.5

3 mM MnCl₂

1 mM EGTA

100 mM NaCl

0,03% CHAPS

2 mM DTT

ATP: variable concentration

1 % (v/v) DMSO

Substrate: PI: 25 µM / PS: 225 µM

PI: L-alpha-phosphatidylinositol

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

PI4K2A: 1.0 µg/ml



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

PI4K2A

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PI4K2A 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 P QID	KYLKSSKYIA	WPLQGQATF	GGGDHPPKSD	PMGHHHHHG	RDSL EVLFQ	240
241	PLAM DETS PL	VSPERAQPPD	YTFPSGSGAH	FPQVPGGAVR	VAAAAGSGPS	PPGSPGH DRE	300
301	RQPLLD RARG	AAAQGQTQTV	AAQAQALAAQ	AAAAAHAAQA	HRERNEFPED	PEFEAVVRQA	360
361	ELAIERCIFP	ERIYQGSSGS	YFVKDPQGRI	IAVFKPKNEE	PYGHLPKWT	KWLQKLCCPC	420
421	CFGRDCLVLN	QGYLSEAGAS	LVDQKLELNI	VPRTKVVYLA	SETFNYS AID	RVKSRGKRLA	480
481	LEKVPKVGQR	FNRIGLPPKV	GSFQLFVEGY	KDADYWLRRF	EAEPLPENTN	RQLLLQFERL	540
541	VLDYIIRNT	DRGNDNWLK	YDCPMDSSSS	RDTDWVVVKE	PVIKVA AIDN	GLAFPLKHPD	600
601	SWRAYPFYWA	WLPQAKVPFS	QEIKDLILPK	ISDPNFVKDL	EEDLYELFKK	DPGFDRGQFH	660
661	KQIAVMRGQI	LNL TQALKDN	KSPLHLVQMP	PVIVETARSH	QRSSSESYTQ	SFQSRKPF FS	720
721	W						780

1-218: GST **Red:** HIS6-tag **Green:** 3C cleavage site **blue:** PI4K2A

PI4K2A wt ¹ amino acid sequence							
1	MDETSPLVSP	ERAQPPDYTF	PSGSGAHFPQ	VPGGAVRVAA	AAGSGPSPPG	SPGHDRERQ P	60
61	LLDRARGAAA	QGQTQTVA AQ	AQALAAQAAA	AAHAAQAHRE	RNEFPEDPEF	EAVVRQAE LA	120
121	IERCIFPERI	YQGSSGSYFV	KDPQGRI IAV	FKPKNEEPYG	HLNPKWTKWL	QKLCCPCCFG	180
181	RDCLVLNQGY	LSEAGASLVD	QKLELNIVPR	TKVVYLASET	FNYS AIDRVK	SRGKRLALEK	240
241	VPKVGQRFNR	IGLPPKVG SF	QLFVEGYKDA	DYWLRRFEAE	PLPENTNRQL	LLQFERLVVL	300
301	DYIIRNTDRG	NDNWLK YDC	PMDSSSRDT	DWVVVKEPVI	KVAAIDNGLA	FPLKHPDSWR	360
361	AYPFYAWLP	OAKVPFSQEI	KDLILPKISD	PNFVKDLEED	LYELFKKDPG	FDRGQFH KQI	420
421	AVMRGQILNL	TQALKDNKSP	LHLVQMP PVI	VETARSHQRS	SSESYTQSFQ	SRKPF FS WW	480

blue: PI4K2A sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_060895.1