Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ PIP5K1A

phosphatidylinositol-4-phosphate 5-kinase type 1 alpha

Recombinant Human Active Lipid Kinase

HGNC Symbol: PIP5K1A

Synonyms: PIP5K1-alpha, PIP5KIalpha, PtdIns(4)P-5-kinase 1 alpha

Lipid Kinase Family: PIP5K
(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.: 1231-0000-1

Lot: 003

Description: Human PIP5K1A, full length, amino acids M_1-H_{500} (as in NCBI/Protein entry AAC50912.1), untagged, expressed in Sf9 insect cells

Product identity: PIP5K1A Lot 003, was confirmed as PIP5K1A by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 56,053 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

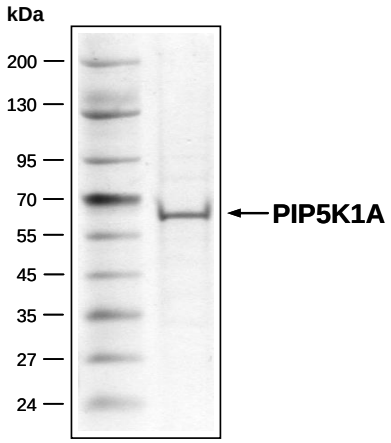
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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

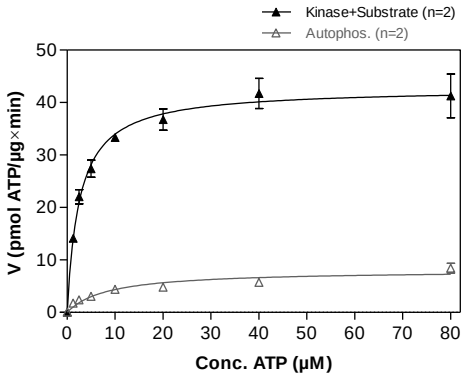
Biochemical Parameters:
Specific kinase activity (P_i transfer): 43 pmol/µg × min
ATP-K_M: 3 µM

PIP5K1A Lot 003:
Coomassie stain



2.0 µg PIP5K1A

PIP5K1A Lot 003:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
• Assay conditions:
60 mM HEPES-NaOH, pH 7.5
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml $PEG_{20.000}$
ATP (variable)
Substrate: PI: 25 µM / PS: 225 µM
PI: L-alpha-phosphatidylinositol
PS: 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-L-Serine])
PIP5K1A: 1 µg/ml

ProQinase™ PIP5K1A

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PIP5K1A Recombinant Fusion Protein Amino Acid Sequence

1	GPLAMGARGR **MASASSGPSS SVGFSSFDPA VPSCTLSSAS GIKRPMASEV PYASGMPIKK**	60
61	**IGHRSVDSSG ETTYKKTTSS ALKGAIQLGI THTVGSLSTK PERDVLMQDF YVVESIFFPS**	120
121	**EGSNLTPAHH YNDFRFKTYA PVAFRYFREL FGIRPDDYLY SLCSEPLIEL CSSGASGSLF**	180
181	**YVSSDDEFII KTVQHKEAEF LQKLLPGYYM NLNQNPRTLL PKFYGLYCVQ AGGKNIRIVV**	240
241	**MNNLLPRSVK MHIKYDLKGS TYKRRASQKE REKPLPTFKD LDFLQDIPDG LFLDADMYNA**	300
301	**LCKTLQRDCL VLQSFKIMDY SLLMSIHNID HAQREPLSSE TQYSVDTRRP APQKALYSTA**	360
361	**MESIQGEARR GGTMETDDHM GGIPARNSKG ERLLLYIGII DILQSYRFVK KLEHSWKALV**	420
421	**HDGDTVSVHR PGFYAERFQR FMCNTVFKKI PCVHLGRPDV LPQTPPLEEI SEGSPIPDPS**	480
481	**FSPLVGETLQ MLTTSTTLEK LEVAESEFTH**	540

1-10: legacy of 3C cleavage **blue**: PIP5K1A

PIP5K1A wt[1] Amino Acid Sequence

1	**MASASSGPSS SVGFSSFDPA VPSCTLSSAS GIKRPMASEV PYASGMPIKK IGHRSVDSSG**	60
61	**ETTYKKTTSS ALKGAIQLGI THTVGSLSTK PERDVLMQDF YVVESIFFPS EGSNLTPAHH**	120
121	**YNDFRFKTYA PVAFRYFREL FGIRPDDYLY SLCSEPLIEL CSSGASGSLF YVSSDDEFII**	180
181	**KTVQHKEAEF LQKLLPGYYM NLNQNPRTLL PKFYGLYCVQ AGGKNIRIVV MNNLLPRSVK**	240
241	**MHIKYDLKGS TYKRRASQKE REKPLPTFKD LDFLQDIPDG LFLDADMYNA LCKTLQRDCL**	300
301	**VLQSFKIMDY SLLMSIHNID HAQREPLSSE TQYSVDTRRP APQKALYSTA MESIQGEARR**	360
361	**GGTMETDDHM GGIPARNSKG ERLLLYIGII DILQSYRFVK KLEHSWKALV HDGDTVSVHR**	420
421	**PGFYAERFQR FMCNTVFKKI PCVHLGRPDV LPQTPPLEEI SEGSPIPDPS FSPLVGETLQ**	480
481	**MLTTSTTLEK LEVAESEFTH**	540

blue: PIP5K1A sequence expressed in recombinant protein

[1]NCBI/Protein accession number AAC50912.1

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