

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Phosphatidylinositol-5-phosphat 4-kinase type II alpha

<u>Enzyme description:-</u>	PIP5K2A [1 - 406]
<u>Clone number:-</u>	DU 12296
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	10 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	49, 737.04 daltons
Average Mass	49, 768.50 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.93
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Hepes/NaOH pH7, 150 mM NaCl, 5 mM DTT, 20% Glycerol, 1 mM Benzamidine, 0.2 mM PMSF	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Kinase Glo
<u>Assay buffer:-</u>	
50 mM Tris, 134 mM KCl, 2 mM DTT, 1 mM EGTA, 10 mM MgCl ₂	
<u>Substrate:-</u>	
PI(5)P diC8	Final concentration: 50 μM

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Clone Data Sheet

PIP5K2A [1 - 406]

Protein PIP5K2A [1 – 406]

Clone number DU 12296

Species Human

Accession number NM_005028.4

Tags N-terminal His(6)

Bacterial expressed protein MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMATPGNLGSSVLASK
TKTKKKHFVAQVKLFRASDPLLSVLMWGVNHSINELSHVQIPVMLMPD
DFKAYSKI KVDNHLFNKENMPSHFKFKEYCPMVFRNLRERFGIDDQDFQ
NSLTRSAPLPNDSQARSGARFHTSYDKRY I IKTITSEDVAEMHNILKKY
HQY IVECHGITLLPQFLGMYRLNVDGVEI YVIVTRNVF SHRLSVYRKYD
LKGSTVAREASDKEKAKELPTLKDNDF INEGQKI YIDDNNKKVFLEKLG
KDVEFLAQLKLM DYSLLVGIHDVERAEQEEVECEENDGEEEGESDGTHP
VGT PPDSPGNTLNS SPPLAPGEFDPNIDVYGIKCHENS PRKEVYFMAI I
DILTHYDAKKAAHA AAKTVKHGAGAEI STVNPEQYSKRFLDF IGHILT

Native sequence Amino acids M1 – T406 (end) of human PIP5K2A.
Residue M35 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage Thrombin (LVPRGS) at residues 14 - 19

Cloning sites *Bam*H1 and *Not*I sites of pET28a

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Complete nucleotide Sequence

ggatccATGGCGACCCCCGGCAACCTAGGGTCCTCTGTCCTGGCGAGCA
AGACCAAGACCAAGAAGAAGCACTTCGTAGCGCAGAAAGTGAAGCTGTT
TCGGGCCAGCGACCCGCTGCTCAGCGTCCTCATGTGGGGGGTAAACCAC
TCGATCAATGAACTGAGCCATGTTCAAATCCCTGTTATGTTGATGCCAG
ATGACTTCAAAGCCTATTCAAAAATAAAGGTGGACAATCACCTTTTTTAA
CAAAGAAAACATGCCGAGCCATTTCAAGTTTAAAGGAATACTGCCCGATG
GTCTTCCGTAACCTGCGGGAGAGGTTTGAATTGATGATCAAGATTTCC
AGAATTCCCTGACCAGGAGCGCACCCCTCCCCAACGACTCCCAGGCCCG
CAGTGGAGCTCGTTTTCACACTTCTACGACAAAAGATACATCATCAAG
ACTATTACCAGTGAAGACGTGGCCGAAATGCACAACATCCTGAAGAAAT
ACCACCAGTACATAGTGGAATGTCATGGGATCACCCCTTCTTCCCAGTT
CTTGGGCATGTACCGGCTTAATGTTGATGGAGTTGAAATATATGTGATA
GTTACAAGAAATGTATTCAGCCACCGTTTGTCTGTGTATAGGAAATACG
ACTTAAAGGGCTCTACAGTGGCTAGAGAAGCTAGTGACAAAGAAAAGGC
CAAAGAACTGCCAACTCTGAAAGATAATGATTTTCATTAATGAGGGCCAA
AAGATTTATATTGATGACAACAACAAGAAGGTCTTCCCTGGAAAACTAA
AAAAGGATGTTGAGTTTCTGGCCCAGCTGAAGCTCATGGACTACAGTCT
GCTGGTGGGAATTCATGATGTGGAGAGAGCCGAACAGGAGGAAGTGGAG
TGTGAGGAGAACGATGGGGAGGAGGAGGGCGAGAGCGATGGCACCCACC
CGGTGGGAACCCCCCAGATAGCCCCGGGAATACACTGAACAGCTCACC
ACCCCTGGCTCCCGGGGAGTTCGATCCGAACATCGACGTCTATGGAATT
AAGTGCCATGAAAACTCGCCTAGGAAGGAGGTGTACTTCATGGCAATTA
TTGACATCCTTACTCATTATGATGCAAAAAAGAAAGCTGCCCATGCTGC
AAAAACTGTTAAACATGGCGCTGGCGCGGAGATCTCCACCGTGAACCCA
GAACAGTATTCAAAGCGCTTTTTGGACTTTATTGGCCACATCTTGACGt
aagcggccgc