Division of Signal Transduction Therapy

Standard Operating Procedure

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

PIP5K2A [1 – 406]

Enzyme description:- PIP5K2A [1 - 406]

Clone number:- DU 12296

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 10 mg/L

Calculated molecular mass:-
Monoisotopic 49, 737.04 daltons
Average Mass 49, 768.50 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.93

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Hepes/NaOH pH7, 150 mM NaCl, 5 mM DTT, 20% Glycerol,
1 mM Benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- ADP Glo

Assay buffer:-
12.5mM Glycine-NaOH (pH 8.5), 50mM KCl, 2.5mM MgCl2, 1mM DTT, 0.25% Na-Cholate

Substrate:-
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	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS**MATPGNLGSSVLASK TKTKKKHFVAQKVKLFRASDPLLSVLMWGVNHSINELSHVQIPVMLMPD DFKAYSKIKVDNHLFNKENMPSHFKFKEYCPMVFRNLRERFGIDDQDFQ NSLTRSAPLPNDSQARSGARFHTSYDKRYIIKTITSEDVAEMHNILKKY HQYIVECHGITLLPQFLGMYRLNVDGVEIYVIVTRNVFSHRLSVYRKYD LKGSTVAREASDKEKAKELPTLKDNDFINEGQKIYIDDNNKKVFLEKLK KDVEFLAQLKLMDYSLLVGIHDVERAEQEEVECEENDGEEEGESDGTHP VGTPPDSPGNTLNSSPPLAPGEFDPNIDVYGIKCHENSPRKEVYFMAII DILTHYDAKKKAAHAAKTVKHGAGAEISTVNPEQYSKRFLDFIGHILT**
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 *Notl*1 sites of pET28a

Complete nucleotide Sequence

ggatccATGGCGACCCCCGGCAACCTAGGGTCCTCTGTCCTGGCGAGCA
AGACCAAGACCAAGAAGAAGCACTTCGTAGCGCAGAAAGTGAAGCTGTT
TCGGGCCAGCGACCCGCTGCTCAGCGTCCTCATGTGGGGGGTAAACCAC
TCGATCAATGAACTGAGCCATGTTCAAATCCCTGTTATGTTGATGCCAG
ATGACTTCAAAGCCTATTCAAAAATAAAGGTGGACAATCACCTTTTTAA
CAAAGAAAACATGCCGAGCCATTTCAAGTTTAAGGAATACTGCCCGATG
GTCTTCCGTAACCTGCGGGAGAGGTTTGGAATTGATGATCAAGATTTCC
AGAATTCCCTGACCAGGAGCGCACCCCTCCCCAACGACTCCCAGGCCCG
CAGTGGAGCTCGTTTTCACACTTCCTACGACAAAAGATACATCATCAAG
ACTATTACCAGTGAAGACGTGGCCGAAATGCACAACATCCTGAAGAAAT
ACCACCAGTACATAGTGGAATGTCATGGGATCACCCTTCTTCCCCAGTT
CTTGGGCATGTACCGGCTTAATGTTGATGGAGTTGAAATATATGTGATA
GTTACAAGAAATGTATTCAGCCACCGTTTGTCTGTGTATAGGAAATACG
ACTTAAAGGGCTCTACAGTGGCTAGAGAAGCTAGTGACAAAGAAAAGGC
CAAAGAACTGCCAACTCTGAAAGATAATGATTTCATTAATGAGGGCCAA
AAGATTTATATTGATGACAACAACAAGAAGGTCTTCCTGGAAAAACTAA
AAAAGGATGTTGAGTTTCTGGCCCAGCTGAAGCTCATGGACTACAGTCT
GCTGGTGGGAATTCATGATGTGGAGAGAGCCGAACAGGAGGAAGTGGAG
TGTGAGGAGAACGATGGGGAGGAGGAGGGCGAGAGCGATGGCACCCACC
CGGTGGGAACCCCCCCAGATAGCCCCGGGAATACACTGAACAGCTCACC
ACCCCTGGCTCCCGGGGAGTTCGATCCGAACATCGACGTCTATGGAATT
AAGTGCCATGAAAACTCGCCTAGGAAGGAGGTGTACTTCATGGCAATTA
TTGACATCCTTACTCATTATGATGCAAAAAAGAAAGCTGCCCATGCTGC
AAAAACTGTTAAACATGGCGCTGGCGCGGAGATCTCCACCGTGAACCCA
GAACAGTATTCAAAGCGCTTTTTGGACTTTATTGGCCACATCTTGACGt
aagcggccgc