Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PI4K2A [1 – 479]

Enzyme description:- PI4K2A [1 - 479]

Clone number:- DU 33414

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 81, 168.41 daltons
Average Mass 81, 219.94 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.76

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:- ADP Glo

Assay Buffer:-
12.5 mM glycine-NaOH pH 8.5, 50 mM KCl, 1 mM DTT, 0.05 % CHAPS, 2.5 mM $MgC1_2$

Substrate:-
PIP2 diC8 Final concentration: 0.05 mM

Clone Data Sheet

PI4K2A [1 - 479]

Protein	PI4K2A [1 - 479]
Clone number	DU 33414
Species	Human
Accession number	NM_018425.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**MDETSPLVSPE** **RAQPPDYTFPSGSGAHFPQVPGGAVRVAAAAGSGPSPPGSPGHDRERQP** **LLDRARGAAAQGQTQTVAAQAQALAAQAAAAAHAAQAHRERNEFPEDPE** **FEAVVRQAELAIERCIFPERIYQGSSGSYFVKDPQGRIIAVFKPKNEEP** **YGHLNPKWTKWLQKLCCPCCFGRDCLVLNQGYLSEAGASLVDQKLELNI** **VPRTKVVYLASETFNYSAIDRVKSRGKRLALEKVPKVGQRFNRIGLPPK** **VGSFQLFVEGYKDADYWLRRFEAEPLPENTNRQLLLQFERLVVLDYIIR** **NTDRGNDNWLIKYDCPMDSSSSRDTDWVVVKEPVIKVAAIDNGLAFPLK** **HPDSWRAYPFYWAWLPQAKVPFSQEIKDLILPKISDPNFVKDLEEDLYE** **LFKKDPGFDRGQFHKQIAVMRGQILNLTQALKDNKSPLHLVQMPPVIVE** **TARSHQRSSSESYTQSFQSRKPFFSWW**
Native sequence	Amino acids M1 – W479 (end) of human PI4K2A. Residue M235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Eco*RI and *Not*1 sites of pGEX 6P-2

Nucleotide Sequence of insert

gaattcATGGACGAGACGAGCCCACTAGTGTCCCCCGAGCGGGCCCAAC
CCCCGGACTACACCTTCCCGTCGGGCTCGGGCGCTCACTTTCCGCAGGT
GCCCGGGGGCGCGGTCCGAGTGGCGGCGGCGGCCGGCTCGGGCCCCTCT
CCGCCGGGCTCGCCGGGCCACGACCGCGAGCGGCAGCCACTGTTGGATC
GGGCCCGGGGCGCGGCGGCCCAGGGCCAGACCCAAACCGTGGCGGCGCA
GGCCCAGGCTCTGGCCGCTCAGGCCGCGGCGGCAGCCCACGCCGCTCAG
GCCCACCGCGAGCGGAACGAGTTCCCGGAGGATCCTGAGTTCGAGGCGG
TGGTGCGGCAGGCCGAGCTGGCCATCGAGCGCTGCATCTTTCCCGAGCG
CATCTACCAGGGCTCCAGCGGAAGCTACTTCGTCAAGGACCCTCAGGGG
AGGATCATTGCTGTCTTCAAACCCAAGAATGAAGAGCCCTATGGGCATC
TTAATCCTAAGTGGACCAAGTGGCTGCAGAAGCTGTGCTGTCCTTGCTG
CTTTGGCCGTGACTGCCTTGTCCTTAACCAGGGCTATCTCTCAGAAGCA
GGGGCCAGCCTGGTGGACCAAAAACTGGAACTCAACATTGTTCCCCGTA
CAAAGGTAGTATACCTGGCCAGTGAGACCTTCAACTATAGTGCCATTGA
CCGAGTGAAGTCCAGGGGCAAGCGGCTTGCACTAGAGAAAGTGCCAAAA
GTTGGACAGCGGTTTAACCGCATCGGGCTACCACCAAAGGTTGGTTCAT
TCCAGCTCTTTGTTGAAGGCTACAAAGATGCAGACTATTGGCTGCGGCG
TTTTGAAGCAGAACCTCTTCCTGAGAACACTAACCGGCAACTACTGCTC
CAGTTTGAGCGGTTGGTGGTGCTGGATTACATCATCCGCAACACTGATC
GAGGCAATGACAACTGGCTGATTAAATATGACTGTCCAATGGATAGTTC
TAGCTCTCGGGACACAGACTGGGTGGTGGTGAAGGAGCCTGTTATCAAG
GTGGCTGCCATAGACAATGGGCTGGCCTTCCCACTGAAGCATCCTGACT
CCTGGAGGGCATATCCTTTTTACTGGGCCTGGTTGCCCCAGGCGAAAGT
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CCTAACTTCGTCAAGGACTTGGAAGAGGACCTATATGAACTCTTCAAGA
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GCGGGGCCAGATCTTAAATCTGACCCAGGCCTTGAAAGACAACAAGAGT
CCCCTGCACCTCGTCCAGATGCCACCTGTGATTGTCGAGACGGCCCGTT
CCCACCAGCGGTCTTCTAGCGAGTCCTACACACAGAGCTTTCAGAGCCG
GAAGCCCTTCTTTTCATGGTGGtaggcggccgc