

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PI3 kinase beta [1 – 1070] / p85 [1 - 724]

<u>Enzyme description:-</u>	PI3 kinase beta [1 – 1070] / p85 [1 - 724]
<u>Clone number:-</u>	DU 5926
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) PI3 kinase alpha No tag for p85
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	3 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	123, 636.62 daltons [PI3kinase beta] and 83, 546.54 daltons [P85]
Average Mass	123, 716.37 daltons [PI3kinase beta] and 83, 598.39 daltons [P85]
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.78 for PI3kinase beta and 5.84 for P85
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50mM Hepes/NaOH pH7.0, 150 mM NaCl, 5 mM DTT, 20 % glycerol	
<u>Storage temperature:-</u>	-70 °C
<u>Assay:-</u>	ADP Glo
<u>Assay buffer:-</u>	
12.5 mM glycine-NaOH pH 8.5, 50 mM KCl, 1 mM DTT, 0.05 % CHAPS, 2.5 mM MgCl ₂	
<u>Substrate:-</u>	
PI(4,5)P ₂ diC8	Final concentration: 0.05 mM

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Clone Data Sheet

PI3 kinase beta [1 – 1070] / p85 [1 - 724]

Protein PI3 kinase beta [1 – 1070] / p85 [1 – 724]

Clone number DU 5926

Species Human

Accession number PI3 kinase beta NM_006219 / p85 NM_181523

Tags N-terminal His(6)

Baculovirus expressed
PI3 kinase beta

MHHHHHMCFSFIMPPAMADILDIWAVDSQIASDGSIPVDFLLPTGIYI
QLEVPREATISYIKQMLWKQVHNYPMFNLLMDIDSYMFACVNQTAVYEE
LEDETRRLCDVRPFLPVLKLVTRSCDPGEKLDISKIGVLIGKGLHEFDSL
KDPEVNEFRKMRKFSEEKILSLVGLSWMDWLKQTYPPPEHEPSIPENLE
DKLYGGKLIVAVHFENCQDVFSFQVSPNMNPIKVNELAIQKRLTIHGKE
DEVSPYDYVLQVSGRVEYVFGDHPLIQFQYIRNCVMNRALPHFILVECC
KIKKMYEQEMIAIEAAINRNSSNLPLPLPPKKTRIISHVWENNNPFQIV
LVKGNKLNTEETVKVHVRAGLFGHTELLCKTIVSSEVSGKNDHIWNEPL
EFDINICDLPRMARLCFAVAVLDKVTKKSTKTINPSKYQTIRKAGKV
HYPVAWVNTMVDFDKGQLRTGDIILHSWSSFPDELEEMLNPMGTVQTNP
YTENATALHVKFPENKKQPYYPFDKIIKAAEIASSDSANVSSRGKG
KFLPVLKEILDRDPLSQLCENEMDLIWTLRQDCREIFPQSLPKLLLSIK
WNKLEDVAQLQALLQIWPKLPPREALELLDFNYPDQYVREYAVGCLRQM
SDEELSQYLLQLVQVLKYEPFLDCALSRFLLERALGNRRIGQFLFWHLR
SEVHIPAVSVQFGVILEAYCRGSGVGHMKVLSKQVEALNKLKTLNSLIKL
NAVKLNRAKGKEAMHTCLKQSAYREALSDLQSPNPCVILSELYVEKCK
YMDSKMKPLWL VYNNKVFGEDSVGVIFKNGDDLQDMLTLQMLRLMDLL
WKEAGLDLRLPYGCLATGDRSGLIEVVSTSETIADIQLNSSNVAAAAA
FNKDALLNLKEYNSGDDLDRATIEFTLSCAGYCVASYVLGIGDRHSDN
IMVKKTGQLFHIDFGHILGNFKSKFGIKRERVPFILTYDFIHVIQOGKT
GNTEKFGRFRQCCEDAYLILRRHGNLFITLFAFMLTAGLPELTSVKDIQ
YLKDSLALGKSEEEALKQFKQKFDEALRESWTTKVNWMAHTVRKDYRS

Native sequence Amino acids M1 – S1070 (end) of human PI3 kinase beta.
PI3 kinase beta Residue M8 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.

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Baculovirus expressed P85

MSAEGYQYRALYDYKKEREEDIDLHLGDILTVNKGSLVALGFSDGQEAR
PEEIGWLNNGYNETTGERGDFPGTYVEYIGRKKISPPTPKPRPPRPLPVA
PGSSKTEADVEQQALTLPDLAEQFAPPDIAPPLLIKLV EAI EKKGLECS
TLYRTQSSSNLAELRQLLD CDTPSVDLEMIDVHVLADAFKRYLLDLPNP
VIPAAVYSEMISLAPEVQSSE EYIQLLKKLIRSPSIPHQYWLTLQYLLK
HFFKLSQTSSKNLLNARVLSEIFSPMLFRFSAASSDNTENLIKVIEILI
STEWNERQPAPALPPKPPKPTTVANNGMNNNMSLQDAEWYWGDISREEV
NEKL RDTADGTFLVRDASTKMHGDYTLTLRKGGNNKLIKIFHRDGKYGF
SDPLTFSSVVELINHYRNESLAQYNPKLDVKLLYPVSKYQQDQVVKEDN
IEAVGKKLHEYNTQFQ EKSREYDRLYE EYTRTSQEIQMKRTAIEAFNET
IKIFEEQCQTQ ERY SKEYIEKFKREGNEKEIQRIMHNYDKLKSRISEII
DSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTQKG
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHHDEKTWNVGSSNRNKAENL
LRGKRDGTFLVRESSKQGCYACSVVVDGEVKHCVINKTATGYGFAEPYN
LYSSLKELVLHYQHTSLVQHND SLNVTLAYPVYAQQRR

Native sequence P85

Amino acids M1 – R724 (end) of human P85.

Cloning sites

*Xho*1 and *Sph*1 (insert 1, PI3 kinase beta) and
*Bam*H1 and *Spe*1 (insert 2, P85) sites of pFastBAC Dual

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Complete nucleotide of sequence of PI3 kinase beta

ATGCATCACCATCACCATCACATGTGCTTCAGTTTCATAATGCCTCCTG
CTATGGCAGACATCCTTGACATCTGGGCGGTGGATTACAGATAGCATC
TGATGGCTCCATACCTGTGGATTTCTTTTGCCCACTGGGATTTATATC
CAGTTGGAGGTACCTCGGGAAGCTACCATTTCTTATATTAAGCAGATGT
TATGGAAGCAAGTTCACAATTACCCAATGTTCAACCTCCTTATGGATAT
TGACTCCTATATGTTTGCATGTGTGAATCAGACTGCTGTATATGAGGAG
CTTGAAGATGAAACACGAAGACTCTGTGATGTCAGACCTTTTCTTCCAG
TTCTCAAATTAGTGACAAGAAGTTGTGACCCAGGGGAAAAATTAGACTC
AAAAATTGGAGTCCTTATAGGAAAAGGTCTGCATGAATTTGATTCCTTG
AAGGATCCTGAAGTAAATGAATTTCAAGAAAAATGCGCAAATTCAGCG
AGGAAAAAATCCTGTCACCTTGTGGGATTGTCTTGGATGGACTGGCTAAA
ACAAACATATCCACCAGAGCATGAACCATCCATCCCTGAAAACCTTAGAA
GATAAACTTTATGGGGGAAAGCTCATCGTAGCTGTTTCAATTTTGAAAAC
GCCAGGACGTGTTTAGCTTTCAAGTGTCTCCTAATATGAATCCTATCAA
AGTAAATGAATTGGCAATCCAAAAACGTTTGACTATTCATGGGAAGGAA
GATGAAGTTAGCCCCTATGATTATGTGTTGCAAGTCAGCGGGAGAGTAG
AATATGTTTTTGGTGATCATCCACTAATTCAGTTCAGTATATCCGGAA
CTGTGTGATGAACAGAGCCCTGCCCCATTTTATACTTGTGGAATGCTGC
AAGATCAAGAAAAATGTATGAACAAGAAATGATTGCCATAGAGGCTGCCA
TAAATCGAAATTCATCTAATCTTCCTCTTCCATTACCACCAAAGAAAAC
ACGAATTATTTCTCATGTTTGGGAAAATAACAACCCTTTCCAAATTGTC
TTGGTTAAGGGAAATAAACTTAACACAGAGGAACTGTAAAAGTTTCATG
TCAGGGCTGGTCTTTTTTCATGGTACTGAGCTCCTGTGTAAAACCATCGT
AAGCTCAGAGGTATCAGGGAAAAATGATCATATTTGGAATGAACCACTG
GAATTTGATATTAATATTTGTGACTTACCAAGAATGGCTCGATTATGTT
TTGCTGTTTATGCAGTTTGGGATAAAGTAAAAACGAAGAAATCAACGAA
AACTATTAATCCCTCTAAATATCAGACCATCAGGAAAGCTGGAAAAGTG
CATTATCCTGTAGCGTGGGTAAATACGATGGTTTTTTGACTTTAAAGGAC
AATTGAGAAGTGGAGACATAATATTACACAGCTGGTCTTTCATTTCTCTGA
TGAAGTCAAGAAATGTTGAATCCAATGGGAAGTGTTCAAACAAATCCA
TATACTGAAAATGCAACAGCTTTGCATGTTAAATTTCCAGAGAATAAAAA
AACAACTTATTATTACCTCCCTTCGATAAGATTATTGAAAAGGCAGC
TGAGATTGCAAGCAGTGATAGTGCTAATGTGTCAAGTCGAGGTGGAAAA
AAGTTTCTTCTCTGTATTGAAAGAAATCTTGGACAGGGATCCCTTGTCTC
AACTGTGTGAAAATGAAATGGATCTTATTTGGACTTTGCGACAAGACTG
CCGAGAGATTTTCCACAATCACTGCCAAAATTACTGCTGTCAATCAAG
TGGAATAAACTTGAGGATGTTGCTCAGCTTCAGGCGCTGCTTCAGATTT
GGCCTAAACTGCCCCCCCCGGGAGGCCCTAGAGCTTCTGGATTTCAACTA
TCCAGACCAGTACGTTTCGAGAATATGCTGTAGGCTGCCTGCGACAGATG
AGTGATGAAGAACTTTCTCAATATCTTTTACAAGTGGTGCAAGTGTTAA
AATATGAGCCTTTTCTTGATTGTGCCCTCTCTAGATTCTTATTAGAAAG
GGCACTTGGTAATCGGAGGATAGGGCAGTTTCTATTTTGGCATCTTAGG
TCAGAAGTGCACATTCCTGCTGTCTCAGTACAATTTGGTGTATCCTTG
AAGCATACTGCCGGGGAAGTGTGGGGCACATGAAAGTGCTTTCTAAGCA
GGTTGAAGCACTCAATAAGTTAAAAACTTTAAATAGTTTAAATCAAACCTG
AATGCCGTGAAGTTAAACAGAGCCAAAGGGAAGGAGGCCATGCATACCT
GTTTAAAAACAGAGTGCTTACCGGGAAGCCCTCTCTGACCTGCAGTCACC

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CCTGAACCCATGTGTTATCCTCTCAGAACTCTATGTTGAAAAGTGCAAA
TACATGGATTCCAAAATGAAGCCTTTGTGGCTGGTATACAATAACAAGG
TATTTGGTGAGGATTCAGTTGGAGTGATTTTTTAAAAATGGTGATGATTT
ACGACAGGATATGTTGACACTCCAAATGTTGCGCTTGATGGATTTACTC
TGGAAAGAAGCTGGTTTGGATCTTCGGATGTTGCCTTATGGCTGTTTAG
CAACAGGAGATCGCTCTGGCCTCATTGAAGTTGTGAGCACCTCTGAAAC
AATTGCTGACATTCAGCTGAACAGTAGCAATGTGGCTGCTGCAGCAGCC
TTCAACAAAGATGCCCTTCTGAACTGGCTTAAAGAATACAACCTCTGGGG
ATGACCTGGACCGAGCCATTGAGGAATTTACACTGTCCTGTGCTGGCTA
CTGTGTAGCTTCTTATGTCCTTGGGATTGGTGACAGACATAGTGACAAC
ATCATGGTCAAAAAAAGCTGGCCAGCTCTTCCACATTGACTTTGGACATA
TTCTTGGAAATTTCAAATCTAAGTTTGGCATTAAAAGGGAGCGAGTGCC
TTTTATTCTTACCTATGATTTTCATCCATGTCATTCAACAAGGAAAAACA
GGAAATACAGAAAAGTTTGGCCGTTCCGCCAGTGTTGTGAGGATGCAT
ATCTGATTTTACGACGGCATGGGAATCTCTTCATCACTCTCTTTGCGCT
GATGTTGACTGCAGGGCTTCCTGAACTCACATCAGTCAAAGATATACAG
TATCTTAAGGACTCTCTTGCATTAGGGAAGAGTGAAGAAGAAGCACTCA
AACAGTTTAAGCAAAAATTTGATGAGGCGCTCAGGGAAAGCTGGACTAC
TAAAGTGAAGTGGATGGCCACACAGTTCGGAAAGACTACAGATCTtaa
gcatgc

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Complete nucleotide of sequence of P85

ATGAGTGCTGAGGGGTACCAGTACAGAGCGCTGTATGATTATAAAAAGG
AAAGAGAAGAAGATATTGACTTGCACTTGGGTGACATATTGACTGTGAA
TAAAGGGTCCTTAGTAGCTCTTGATTGAGTGATGGACAGGAAGCCAGG
CCTGAAGAAATTGGCTGGTTAAATGGCTATAATGAAACCACAGGGGAAA
GGGGGGACTTTCCGGGAACCTTACGTAGAATATATTGGAAGGAAAAAAT
CTCGCCTCCACACCAAAGCCCCGGCCACCTCGGCCTCTTCCTGTTGCA
CCAGGTTCTTCGAAAACCTGAAGCAGATGTTGAACAACAAGCTTTGACTC
TCCCGGATCTTGCAGAGCAGTTTGCCCCCTCCTGACATTGCCCCGCCTCT
TCTTATCAAGCTCGTGGAGCCATTGAAAAGAAAGGTCTGGAATGTTCA
ACTCTATACAGAACACAGAGCTCCAGCAACCTGGCAGAATTACGACAGC
TTCTTGATTGTGATACACCCTCCGTGGACTTGGAATGATCGATGTGCA
CGTTTTGGCTGACGCTTTCAAACGCTATCTCCTGGACTTACCAAATCCT
GTCATTCCAGCAGCCGTTTACAGTGAAATGATTTCTTTAGCTCCAGAAG
TACAAAGCTCCGAAGAATATATTGAGCTATTGAAGAAGCTTATTAGGTC
GCCTAGCATACCTCATCAGTATTGGCTTACGCTTCAGTATTTGTTAAAA
CATTTCTTCAAGCTCTCTCAAACCTCCAGCAAAAATCTGTTGAATGCAA
GAGTACTCTCTGAAATTTTCAGCCCTATGCTTTTCAGATTCTCAGCAGC
CAGCTCTGATAATACTGAAAACCTCATAAAAGTTATAGAAATTTTAATC
TCAACTGAATGGAATGAACGACAGCCTGCACCAGCACTGCCTCCTAAAC
CACCAAACCTACTACTGTAGCCAACAACGGTATGAATAACAATATGTC
CTTACAAGATGCTGAATGGTACTGGGGAGATATCTCGAGGGAAGAAGTG
AATGAAAACTTCGAGATACAGCAGACGGGACCTTTTTTGGTACGAGATG
CGTCTACTAAAATGCATGGTGATTATACTCTTACACTAAGGAAAGGGG
AAATAACAAATTAATCAAAATATTTTCATCGAGATGGGAAATATGGCTTC
TCTGACCCATTAACCTTCAGTTCTGTGGTTGAATTAATAAACCACTACC
GGAATGAATCTCTAGCTCAGTATAATCCCAAATTGGATGTGAAATTACT
TTATCCAGTATCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAAT
ATTGAAGCTGTAGGGAAAAAATTACATGAATATAACACTCAGTTTCAAG
AAAAAAGTCGAGAATATGATAGATTATATGAAGAATATACCCGCACATC
CCAGGAAATCCAAATGAAAAGGACAGCTATTGAAGCATTTAATGAAACC
ATAAAAATATTTGAAGAACAGTGCCAGACCCAAGAGCGGTACAGCAAAG
AATACATAGAAAAGTTTAAACGTGAAGGCAATGAGAAAGAAATACAAAG
GATTATGCATAATTATGATAAGTTGAAGTCTCGAATCAGTGAAATTATT
GACAGTAGAAGAAGATTGGAAGAAGACTTGAAGAAGCAGGCAGCTGAGT
ATCGAGAAATTGACAAACGTATGAACAGCATTAACCAGACCTTATCCA
GCTGAGAAAGACGAGAGACCAATACTTGATGTGGTTGACTCAAAAAGGT
GTTTCGGCAAAGAAGTTGAACGAGTGGTTGGGCAATGAAAACACTGAAG
ACCAATATTTCACTGGTGGAAGATGATGAAGATTTGCCCCATCATGATGA
GAAGACATGGAATGTTGGAAGCAGCAACCGAAACAAAGCTGAAAACCTG
TTGCGAGGGAAGCGAGATGGCACTTTTCTTGTCCGGGAGAGCAGTAAAC
AGGGCTGCTATGCCTGCTCTGTAGTGGTGGACGGCGAAGTAAAGCATTG
TGTCATAAAACAAAACAGCAACTGGCTATGGCTTTGCCGAGCCCTATAAC
TTGTACAGCTCTCTGAAAGAACTGGTGCTACATTACCAACACACCTCCC
TTGTGCAGCACAACGACTCCCTCAATGTCACACTAGCCTACCCAGTATA
TGCACAGCAGAGGCGatgaactagt