

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>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**

MHHHHHMCFSFIMPPAMADILDIAVDSQIASDGSIPVDFLLPTGIYI
QLEVPREATISYIKQMLWKQVHNYPMFNLLMDIDSYMFACVNQTAVYEE
LEDETRRLCDVRPFLPVLKLVTRSCDPGEKLDISKIGVLIGKGLHEFDSL
KDPEVNEFRKMRKFSEEKILSLVGLSWMDWLKQTYPPPEHEPSIPENLE
DKLYGGKLI VAVHFENCQDVFSFQVSPNMNPIKVNELAIQKRLTIHGKE
DEVSPYDYVLQVSGRVEYVFGDHP LIQFOYIRNCVMNRALPHFILVECC
KIKKMYEQEMIAIEAAINRNSSNLPLPLPKKTRIISHVWENNNPFQIV
LVKGNKLNTEETVKVHVRAGLFGHTELLCKTIVSSEVSGKNDHIWNEPL
EFDINICDLPRMARLCFAVYAVLDKVKTKKSTKTINPSKYQTIKAGKV
HYPVAWVNTMVDFDKGQLRTGDIILHSWSSFPDELEEMLNPMGTVQTNP
YTENATALHVKFPENKKQPYYPFDKIEKAAEIASSDSANVSSRGGK
KFLPVLKEILDRDPLSQLCENEMDLIWTLRQDCREIFPQSLPKLLLSIK
WNKLEDVAQLQALLQIWPKLPPREALELLDFNYPDOYVREYAVGCLRQM
SDEELSQYLLQLVQVLKYEPFLDCALSRFLLERALGNRRIGQFLFWHLR
SEVHIPAVSVQFGVILEAYCRGSGVGHMKVLSKQVEALNKLKTLNSLIKL
NAVKLNRAKGKEAMHTCLKQSAYREALSDLOSPLNPCVILSELYVEKCK
YMDSKMKPLWL VYNNKVFGE DSVGVIFKNGDDL RQDMLTLQMLRLMDLL
WKEAGLDLRLMPYGCLATGDRSGLIEVVSTSETIADIQLNSSNVAAAAA
FNKDALLNLKEYNSGDDL DRAIEEFTLSCAGYCVASYVLGIGDRHSDN
IMVKKTGQLFHIDFGHILGNFKSKFGIKRERVPFILTYDFIHVIQOGKT
GNTEKFGRFRQCCEDAYLILRRHGNLFIITL FALMLTAGLPELTSVKDIO
YLKDSLALGKSEEEALKQFKQKFDEALRESWTTKVNWMAHTVRKDYRS

**Native sequence
PI3 kinase beta**

Amino acids M1 – S1070 (end) of human 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
PEEIGWLNGYNETTGERGDFPGTYVEYIGRKKISPPTPKPRPPRPLPVA
PGSSKTEADVEQQALTLPDLAEQFAPPDIAPPLLIKLV EAI EKKGLECS
TLYRTQSSSNLAELRQLLDCTPSVDLEMIDVHVLADAFKRYLLDLPNP
VIPAAVYSEMISLAPEVQSSEYIQLLKKLIRSPSIPHQYWLTLQYLLK
HFFKLSQTSSKNLLNARVLSEIFSPMLFRFSAASSDNTENLIKVIEILI
STEWNERQPAPALPPKPPKPTTVANNGMNNMSLQDAEWYWGDISREEV
NEKLRDTADGTFLVRDASTKMHGDYTLTLRKGGNNKLIKIFHRDGKYGF
SDPLTFSSVVELINHYRNESLAQYNPKLDVKLLYPVSKYQQDQVVKEDN
IEAVGKKLHEYNTQFOEKSREYDRLYEEYTRTSQEIOMKRTAIEAFNET
IKIFEEQCQTOERYKEYIEKFKREGNEKEIQRIMHNYDKLKSRISEII
DSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTQKG
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHHDEKTWNVGSSNRNKAENL
LRGKRDGTFLVRESSKQGCYACSVVDGEVKHCVINKTATGYGFAEPYN
LYSSLKELVLHYQHTSLVQHNDLNVTLAYPVYAQQRR

**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
TGACTCCTATATGTTTTGCATGTGTGAATCAGACTGCTGTATATGAGGAG
CTTGAAGATGAAACACGAAGACTCTGTGATGTCAGACCTTTTCTTCCAG
TTCTCAAATTAGTGACAAGAAGTTGTGACCCAGGGGAAAAATTAGACTC
AAAAATTGGAGTCTTATAGGAAAAGGTCTGCATGAATTTGATTCCTTG
AAGGATCCTGAAGTAAATGAATTTGGAAGAAAAATGCGCAAATTCAGCG
AGGAAAAAATCCTGTCACCTTGTGGGATTGTCTTGGATGGACTGGCTAAA
ACAAACATATCCACCAGAGCATGAACCATCCATCCCTGAAAACCTAGAA
GATAAACTTTATGGGGAAAGCTCATCGTAGCTGTTCATTTTGAAAACCT
GCCAGGACGTGTTTAGCTTTCAAGTGTCTCCTAATATGAATCCTATCAA
AGTAAATGAATTGGCAATCCAAAAACGTTTGACTATTCATGGGAAGGAA
GATGAAGTTAGCCCCTATGATTATGTGTTGCAAGTCAGCGGGAGAGTAG
AATATGTTTTTGGTGATCATCCACTAATTCAGTTCAGTATATCCGGAA
CTGTGTGATGAACAGAGCCCTGCCCATTTTATACTTGTGGAATGCTGC
AAGATCAAGAAAAATGTATGAACAAGAAATGATTGCCATAGAGGCTGCCA
TAAATCGAAATTCATCTAATCTTCTCCTTCCATTACCACCAAAGAAAAC
ACGAATTATTTCTCATGTTTGGGAAAATAACAACCCTTTCCAAATTGTC
TTGGTTAAGGGAAAATAAATTAACACAGAGGAAACTGTAAAAGTTCATG
TCAGGGCTGGTCTTTTTTCATGGTACTGAGCTCCTGTGTAACCATCGT
AAGCTCAGAGGTATCAGGGAAAAATGATCATATTTGGAATGAACCACTG
GAATTTGATATTAATATTTGTGACTTACCAAGAATGGCTCGATTATGTT
TTGCTGTTTATGCAGTTTTGGATAAAGTAAAAACGAAGAAATCAACGAA
AACTATTAATCCCTCTAAATATCAGACCATCAGGAAAGCTGGAAAAGTG
CATTATCCTGTAGCGTGGGTAAATACGATGGTTTTTTGACTTTAAAGGAC
AATTGAGAACTGGAGACATAATATTACACAGCTGGTCTTCATTTCTCTGA
TGAATCGAAGAAATGTTGAATCCAATGGGAACTGTTCAAACAAATCCA
TATACTGAAAATGCAACAGCTTTGCATGTTAAATTTCCAGAGAATAAAA
AACAACTTATTATTACCTCCCTTCGATAAGATTATTGAAAAGGCAGC
TGAGATTGCAAGCAGTGATAGTGCTAATGTGTCAAGTCGAGGTGGAAAA
AAGTTTTCTTCTGTATTGAAAGAAATCTTGGACAGGGATCCCTTGTCTC
AACTGTGTGAAAATGAAATGGATCTTATTTGGACTTTGCGACAAGACTG
CCGAGAGATTTTCCACAATCACTGCCAAAATTACTGCTGTCAATCAAG
TGGAATAAACTTGAGGATGTTGCTCAGCTTCAGGCGCTGCTTCAGATTT
GGCCTAAACTGCCCCCCGGGAGGCCCTAGAGCTTCTGGATTTCAACTA
TCCAGACCAGTACGTTTCGAGAATATGCTGTAGGCTGCCTGCGACAGATG
AGTGATGAAGAACTTTCTCAATATCTTTTACAACCTGGTGAAGTGTAA
AATATGAGCCTTTTCTTGATTGTGCCCTCTCTAGATTCCTATTAGAAAG
GGCACTTGGTAATCGGAGGATAGGGCAGTTTTCTATTTTGGCATCTTAGG
TCAGAAGTGCACATTCCTGCTGTCTCAGTACAATTTGGTGTTCATCCTTG
AAGCATACTGCCGGGGAAGTGTGGGGCACATGAAAGTGCTTTCTAAGCA
GGTTGAAGCACTCAATAAGTTAAAAACTTTAAATAGTTTAAATCAAACCTG
AATGCCGTGAAGTTAAACAGAGCCAAAGGGAAGGAGGCCATGCATACCT
GTTTAAAACAGAGTGCTTACCGGGAAGCCCTCTCTGACCTGCAGTCACC

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CCTGAACCCATGTGTTATCCTCTCAGAACTCTATGTTGAAAAGTGCAAA
TACATGGATTCCAAAATGAAGCCTTTGTGGCTGGTATAACAATAACAAGG
TATTTGGTGAGGATTCAGTTGGAGTGATTTTTTAAAAATGGTGATGATTT
ACGACAGGATATGTTGACACTCCAATGTTGCGCTTGATGGATTTACTC
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
AAAGAGAAGAAGATATTGACTTGCACCTTGGGTGACATATTGACTGTGAA
TAAAGGGTCCTTAGTAGCTCTTGGATTCAAGTATGGACAGGAAGCCAGG
CCTGAAGAAATTGGCTGGTTAAATGGCTATAATGAAACCACAGGGGAAA
GGGGGGACTTTCCGGGAACCTTACGTAGAAATATATTGGAAGGAAAAAAT
CTCGCCTCCACACCAAAGCCCCGGCCACCTCGGCCTCTTCCTGTTGCA
CCAGGTTCTTCGAAAACCTGAAGCAGATGTTGAACAACAAGCTTTGACTC
TCCCGGATCTTGCAGAGCAGTTTGCCCCCTCCTGACATTGCCCCGCCTCT
TCTTATCAAGCTCGTGGAAAGCCATTGAAAAGAAAGGTCTGGAATGTTCA
ACTCTATACAGAACACAGAGCTCCAGCAACCTGGCAGAATTACGACAGC
TTCTTGATTGTGATACACCCTCCGTGGACTTGGAAATGATCGATGTGCA
CGTTTTGGCTGACGCTTTCAAACGCTATCTCCTGGACTTACCAAATCCT
GTCATTCCAGCAGCCGTTTACAGTGAATGATTTCTTTAGCTCCAGAAG
TACAAAGCTCCGAAGAATATATTCAGCTATTGAAGAAGCTTATTAGGTC
GCCTAGCATACCTCATCAGTATTGGCTTACGCTTACAGTATTTGTTAAAA
CATTTCTTCAAGCTCTCTCAAACCTCCAGCAAAAATCTGTTGAATGCAA
GAGTACTCTCTGAAATTTTCAGCCCTATGCTTTTCAGATTCTCAGCAGC
CAGCTCTGATAATACTGAAAACCTCATAAAAGTTATAGAAATTTTAATC
TCAACTGAATGGAATGAACGACAGCCTGCACCAGCACTGCCTCCTAAAC
CACAAAACCTACTACTGTAGCCAACAACGGTATGAATAACAATATGTC
CTTACAAGATGCTGAATGGTACTGGGGAGATATCTCGAGGGAAGAAGTG
AATGAAAACTTCGAGATACAGCAGACGGGACCTTTTTTGGTACGAGATG
CGTCTACTAAAATGCATGGTGATTATACTCTTACACTAAGGAAAGGGG
AAATAACAAATTAATCAAAATATTTTCATCGAGATGGGAAATATGGCTTC
TCTGACCCATTAACCTTCAGTTCTGTGGTTGAATTAATAAACCCTACC
GGAATGAATCTCTAGCTCAGTATAATCCCAAATGGATGTGAAATTACT
TTATCCAGTATCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAAT
ATTGAAGCTGTAGGGAAAAAATTACATGAATATAACACTCAGTTTCAAG
AAAAAAGTCGAGAATATGATAGATTATATGAAGAATATACCCGCACATC
CCAGGAAATCCAAATGAAAAGGACAGCTATTGAAGCATTTAATGAAACC
ATAAAAATATTTGAAGAACAGTGCCAGACCCAAGAGCGGTACAGCAAAG
AATACATAGAAAAGTTTAAACGTGAAGGCAATGAGAAAAGAAATACAAAG
GATTATGCATAATTATGATAAGTTGAAGTCTCGAATCAGTGAAATTATT
GACAGTAGAAGAAGATTGGAAGAAGACTTGAAGAAGCAGGCAGCTGAGT
ATCGAGAAATTGACAAACGTATGAACAGCATTAAACCAGACCTTATCCA
GCTGAGAAAGACGAGAGACCAATACTTGTGTTGGTTGACTCAAAAAGGT
GTTTCGGCAAAGAAGTTGAACGAGTGGTTGGGCAATGAAAACACTGAAG
ACCAATATTTCACTGGTGAAGATGATGAAGATTTGCCCATCATGATGA
GAAGACATGGAATGTTGGAAGCAGCAACCGAAACAAAGCTGAAAACCTG
TTGCGAGGGAAGCGAGATGGCACTTTTCTTGTCCGGGAGAGCAGTAAAC
AGGGCTGCTATGCCTGCTCTGTAGTGGTGGACGGCGAAGTAAAGCATTG
TGTCATAAACAAAACAGCAACTGGCTATGGCTTTGCCGAGCCCTATAAC
TTGTACAGCTCTCTGAAAGAACTGGTGTACATTACCAACACACCTCCC
TTGTGCAGCACAACGACTCCCTCAATGTCACACTAGCCTACCCAGTATA
TGCACAGCAGAGGCGatgaactagt