

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PI3 kinase alpha [2 – 1068] / p85 [1 - 724]

<u>Enzyme description:-</u>	PI3 kinase alpha [2 – 1068] / p85 [1 - 724]
<u>Clone number:-</u>	DU 1468
<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	125, 025.8 daltons [PI3kinase alpha] and 83, 546.54 daltons [P85]
Average Mass	125, 106.94 daltons [PI3kinase alpha] and 83, 598.39 daltons [P85]
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.94 for PI3kinase alpha 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>	
12.5mM Glycine-NaOH (pH 8.5), 50mM KCl, 2.5mM MgCl ₂ , 1mM DTT, 0.05% CHAPS	
<u>Substrate:-</u>	
PI(4,5)P ₂ diC8	Final concentration: 50 µM

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Clone Data Sheet

PI3 kinase alpha [2 – 1068] / p85 [1 - 724]

Protein PI3 kinase alpha [2 – 1068] / p85 [1 – 724]

Clone number DU 1468

Species Human

Accession number PI3 kinase alpha U79143 / p85 NM_181523

Tags N-terminal His(6)

Baculovirus expressed
PI3 kinase alpha

MHHHHHHPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLI
TIKHELFKEARKYPLHQLLQDESSYIFVSVTQEAEREFFDETTRLCDL
RLFQPFLLKVIIEPVGNREEKILNREIGFAIGMPVCEFDVMKDPEVQDFRR
NILNVCKEAVDLRDLNSPHSRAMYVYPNVESSELPKHIYNKLDKGQI
IVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIKKKTRSMLLSSEQL
KLCVLEYQGGYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNMLM
AKESLYSQLPMDCFMTPSYSRRISTATPYMNGETSTKSLWVINSALRIK
ILCATYVNVNIRDIDKIYVRTGIYHGGEPLCDNVNTQRPVPCSNPRWNEW
LNYDIYIPDLPRARLCLCSVKGKRGAKKEHCPLAWGNINLFDYTD
LVSGKMALNLWPVPHGLEDLLNPIGVTGSNPNKETPCLELEFDWFSSVV
KFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKA
ISTRDPLSEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQ
MYCLVKDWPPIKPEQAMELLDCNYPDPMVRGFAVRCLEKYLTDDKLSQY
LIQLVQVLKYEQYLDNLLVRFLKALTNQRIGHFFFWHLKSEMHNKTV
SQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLTDILKQEKKDETQK
VQMKFLVEQMRRPDFMDALQGFSLNPAHQGLNLRLEECRIMSSAKRP
LWLNWENPDIMSELLFQNEIIFKNGDDLQDMLTLQIIRIMENIWQNO
GLDLRMLPYGCLSIGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNSHT
LHQWLKDKNGEYDAIDLFTRSCAGYCVATFILGIGDRHNSNIMVKD
DGQLFHIDFGHFLDHKKKKFGYKRERVPFVLTQDFLIVISKGAQECTKT
REFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIR
KTLALDKTEQEALEYFMKQMNDAHGGWTTKMDWIFHTIKQHALN

Native sequence Amino acids P2 – N1068 (end) of human PI3 kinase alpha.
PI3 kinase alpha Residue P8 of the fusion protein is equivalent to P2 of the native enzyme.
The His(6) tag is located at residues 2 – 7.

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Baculovirus expressed P85

MSAEGYQYRALYDYKKEREEDIDLHLGDILTVNKGSLVALGFSDGQEAR
PEEIGWLNNGYNETTGERGDFPGTYVEYIGRKKISPPTPKPRPPRPLPVA
PGSSKTEADVEQQALTLPDLAEQFAPPDIAPELLIKLVEAIEKKGLECS
TLYRTQSSSNLAELRQLLDCTPSVDLEMIDVHVLADAFKRYLLDLPNP
VIPAAVYSEMISLAPEVQSSEYIQLLKKLIRSPSIPHQYWLTLQYLLK
HFFKLSQTSSKNLLNARVLSEIFSPMLFRFSAASSDNTENLIKVIEILI
STEWNERQPAPALPPKPPKPTTVANNGMNNNMSLQDAEWYWGDISREEV
NEKLRTADGTFLVRDASTKMHGDYTLTLRKGGNNKLIKIFHRDGKYGF
SDPLTFSSVVELINHYRNESLAQYNPKLDVKLLYPVSKYQQDQVVKEDN
IEAVGKKLHEYNTQFQEKSSREYDRLYEEYTRTSQEIOMKRTAIEAFNET
IKIFEEQCQTOERYKEYIEKFKREGNEKEIQRIMHNYDKLKSRISEII
DSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTQKG
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHHDEKTWNVGSSNRNKAENL
LRGKRDGTFLVRESSKQGCYACSVVVDGEVKHCVINKTATGYGFAEPYN
LYSSLKELVLHYQHTSLVQHNDNLNVTLAYPVYAQQRR

Native sequence P85

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

Cloning sites

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

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**Complete
nucleotide
of sequence of PI3
kinase alpha**

ATGCACCATCACCATCACCATCCTCCAAGACCATCATCAGGTGAACTGT
GGGGCATCCACTTGATGCCCCCAAGAATCCTAGTAGAATGTTTACTACC
AAATGGAATGATAGTGACTTTTAGAATGCCTCCGTGAGGCTACATTAATA
ACCATAAAGCATGAACTATTTAAAGAAGCAAGAAAATACCCCTCCATC
AACTTCTTCAAGATGAATCTTCTTACATTTTTCGTAAGTGTTACTCAAGA
AGCAGAAAGGGAAGAATTTTTTGTATGAAACAAGACGACTTTGTGACCTT
CGGCTTTTTCAACCCTTTTTTAAAAGTAATTGAACCAGTAGGCAACCGTG
AAGAAAAGATCCTCAATCGAGAAATTGGTTTTGTCTATCGGCATGCCAGT
GTGTGAATTTGATATGGTTAAAGATCCAGAAGTACAGGACTTCCGAAGA
AATATTCTGAACGTTTTGTAAAGAAGCTGTGGATCTTAGGGACCTCAATT
CACCTCATAGTAGAGCAATGTATGTCTATCCTCCAAATGTAGAATCTTC
ACCAGAATTGCCAAAGCACATATATAATAAATTAGATAAAGGGCAAATA
ATAGTGGTGATCTGGGTAATAGTTTCTCCAAATAATGACAAGCAGAAGT
ATACTCTGAAAATCAACCATGACTGTGTACCAGAACAAGTAATTGCTGA
AGCAATCAGGAAAAAACTCGAAGTATGTTGCTATCCTCTGAACAACCTA
AACTCTGTGTTTTAGAAATATCAGGGCAAGTATATTTTAAAAGTGTGTG
GATGTGATGAATACTTCCTAGAAAAATATCCTCTGAGTCAGTATAAGTA
TATAAGAAGCTGTATAATGCTTGGGAGGATGCCAATTTGATGTTGATG
GCTAAAGAAAGCCTTTATTCTCAACTGCCAATGGACTGTTTTACAATGC
CATCTTATTCCAGACGCATTTCCACAGCTACACCATATATGAATGGAGA
AACATCTACAAAATCCCTTTGGGTTATAAATAGTGCACTCAGAATAAAA
ATTCTTTGTGCAACCTACGTGAATGTAAATATTTCGAGACATTGATAAGA
TCTATGTTTCGAACAGGTATCTACCATGGAGGAGAACCCTTATGTGACAA
TGTGAACACTCAAAGAGTACCTTGTTCCAATCCCAGGTGGAATGAATGG
CTGAATTATGATATATACATTCCCTGATCTTCCTCGTGCTGCTCGACTTT
GCCTTTCCATTTGCTCTGTAAAGGCCGAAAGGGTGCTAAAGAGGAACA
CTGTCCATTGGCATGGGGAAATATAAACTTGTTTGATTACACAGACACT
CTAGTATCTGGAAAAATGGCTTTGAATCTTTGGCCAGTACCTCATGGAT
TAGAAGATTTGCTGAACCTATTGGTGTTACTGGATCAAATCCAAATAA
AGAACTCCATGCTTAGAGTTGGAGTTTGAAGTTCAGCAGTGTGGTA
AAGTTCCCAGATATGTCAGTGATTGAAGAGCATGCCAATTGGTCTGTAT
CCCGAGAAGCAGGATTTAGCTATTCCCACGCAGGACTGAGTAACAGACT
AGCTAGAGACAATGAATTAAGGGAAAAATGACAAAGAACAGCTCAAAGCA
ATTTCTACACGAGATCCTCTCTCTGAAATCACTGAGCAGGAGAAAGATT
TTCTATGGAGTCACAGACACTATTGTGTAAGTATCCCCGAAATCTTACC
CAAATTGCTTCTGTCTGTAAATGGAATTCTAGAGATGAAGTAGCCCAG
ATGTATTGCTTGGTAAAAGATTGGCCTCCAATCAAACCTGAACAGGCTA
TGGAACCTTCTGGACTGTAATTACCCAGATCCTATGGTTTCGAGGTTTTGC
TGTTCCGGTGCTTGGAAAAATATTTAACAGATGACAACTTTCTCAGTAT
TTAATTACAGCTAGTACAGGTCCTAAATATGAACAATATTTGGATAACT
TGCTTGTGAGATTTTTACTGAAGAAAGCATTGACTAATCAAAGGATTGG
GCACTTTTTCTTTTGGCATTAAAATCTGAGATGCACAATAAAACAGTT
AGCCAGAGGTTTGGCCTGCTTTTGGAGTCCTATTGTCTGTCATGTGGGA
TGTATTTGAAGCACCTGAATAGGCAAGTCGAGGCAATGGAAAAGCTCAT
TAACTTAACTGACATTCTCAAACAGGAGAAGAAGGATGAAACACAAAAG
GTACAGATGAAGTTTTTAGTTGAGCAAATGAGGCGACCAGATTTTCATGG
ATGCTCTACAGGGCTTTCTGTCTCCTCTAAACCTGCTCATCAACTAGG

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AAACCTCAGGCTTGAAGAGTGTCGAATTATGTCCTCTGCAAAAAGGCCA
CTGTGGTTGAATTGGGAGAACCCAGACATCATGTCAGAGTTACTGTTTC
AGAACAATGAGATCATCTTTAAAAATGGGGATGATTTACGGCAAGATAT
GCTAACACTTCAAATTATTTCGTATTATGGAAAATATCTGGCAAAATCAA
GGTCTTGATCTTCGAATGTTACCTTATGGTTGTCTGTCAATCGGTGACT
GTGTGGGACTTATTGAGGTGGTGCGAAATTCCTCACACTATTATGCAAAT
TCAGTGCAAAGGCGGCTTGAAAGGTGCACTGCAGTTCAACAGCCACACA
CTACATCAGTGGCTCAAAGACAAGAACAAAGGAGAAATATATGATGCAG
CCATTGACCTGTTTACACGTTTCATGTGCTGGATACTGTGTAGCTACCTT
CATTTTGGGAATTGGAGATCGTCACAATAGTAACATCATGGTGAAAGAC
GATGGACAACCTGTTTCATATAGATTTTGGACACTTTTGGATCACAAGA
AGAAAAAATTTGGTTATAAACGAGAACGTGTGCCATTTGTTTTGACACA
GGATTTCTTAATAGTGATTAGTAAAGGAGCCCCAAGAATGCACAAAGACA
AGAGAATTTGAGAGGTTTCAGGAGATGTGTTACAAGGCTTATCTAGCTA
TTCGACAGCATGCCAATCTCTTCATAAATCTTTTCTCAATGATGCTTGG
CTCTGGAATGCCAGAACTACAATCTTTTGATGACATTGCATACATTCTGA
AAGACCCTAGCCTTAGATAAAACTGAGCAAGAGGCTTTGGAGTATTTCA
TGAAACAAATGAATGATGCACATCATGGTGGCTGGACAACAAAAATGGA
TTGGATCTTCCACACAATTAAACAGCATGCATTGAACTGAggtacc

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Complete nucleotide of sequence of P85

ATGAGTGCTGAGGGGTACCAGTACAGAGCGCTGTATGATTATAAAAAGG
AAAGAGAAGAAGATATTGACTTGCACTTGGGTGACATATTGACTGTGAA
TAAAGGGTCCTTAGTAGCTCTTGATTGAGTGATGGACAGGAAGCCAGG
CCTGAAGAAATTGGCTGGTTAAATGGCTATAATGAAACCACAGGGGAAA
GGGGGGACTTTCCGGGAACCTTACGTAGAATATATTGGAAGGAAAAAAT
CTCGCCTCCCACACCAAAGCCCCGGCCACCTCGGCCTCTTCCTGTTGCA
CCAGGTTCTTCGAAAACCTGAAGCAGATGTTGAACAACAAGCTTTGACTC
TCCCGGATCTTGCAGAGCAGTTTGCCCCCTCCTGACATTGCCCCGCCTCT
TCTTATCAAGCTCGTGGAAGCCATTGAAAAGAAAGGTCTGGAATGTTCA
ACTCTATACAGAACACAGAGCTCCAGCAACCTGGCAGAATTACGACAGC
TTCTTGATTGTGATACACCCTCCGTGGACTTGGAATGATCGATGTGCA
CGTTTTGGCTGACGCTTTCAAACGCTATCTCCTGGACTTACCAAATCCT
GTCATTCCAGCAGCCGTTTACAGTGAAATGATTTCTTTAGCTCCAGAAG
TACAAAGCTCCGAAGAATATATTGAGCTATTGAAGAAGCTTATTAGGTC
GCCTAGCATACCTCATCAGTATTGGCTTACGCTTCAGTATTTGTTAAAA
CATTTCTTCAAGCTCTCTCAAACCTCCAGCAAAAATCTGTTGAATGCAA
GAGTACTCTCTGAAATTTTTCAGCCCTATGCTTTTTCAGATTCTCAGCAGC
CAGCTCTGATAATACTGAAAACCTCATAAAAGTTATAGAAATTTTAATC
TCAACTGAATGGAATGAACGACAGCCTGCACCAGCACTGCCTCCTAAAC
CACCAAACCTACTACTGTAGCCAACAACGGTATGAATAACAATATGTC
CTTACAAGATGCTGAATGGTACTGGGGAGATATCTCGAGGGAAGAAGTG
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CGTCTACTAAAATGCATGGTGATTATACTCTTACACTAAGGAAAGGGG
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TCTGACCCATTAACCTTCAGTTCTGTGGTTGAATTAATAAACCACTACC
GGAATGAATCTCTAGCTCAGTATAATCCCAAATTGGATGTGAAATTACT
TTATCCAGTATCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAAT
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GATTATGCATAATTATGATAAGTTGAAGTCTCGAATCAGTGAAATTATT
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ATCGAGAAATTGACAAACGTATGAACAGCATTAACCAGACCTTATCCA
GCTGAGAAAGACGAGAGACCAATACTTGATGTGGTTGACTCAAAAAGGT
GTTTCGGCAAAAGAAGTTGAACGAGTGGTTGGGCAATGAAAACACTGAAG
ACCAATATTTCACTGGTGGAAGATGATGAAGATTTGCCCATCATGATGA
GAAGACATGGAATGTTGGAAGCAGCAACCGAAACAAAGCTGAAAACCTG
TTGCGAGGGAAGCGAGATGGCACTTTTCTTGTCGGGGAGAGCAGTAAAC
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TTGTGCAGCACAACGACTCCCTCAATGTCACACTAGCCTACCCAGTATA
TGCACAGCAGAGGCGAtgaactagt