

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>	
	12.5 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 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**

MHHHHHPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLI
TIKHELKFKEARKYPLHQLLQDESSYIFVSVTQEAEREFFDETRRLCDL
RLFQPFVKVIEPVGNRREEKILNREIGFAIGMPVCEFDLVKDEPVQDFRR
NILNVCKEAVDLRDLNSPHSRAMYVYPPNVESPELPHKIYNKLDKGOI
IVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIKKKTRSMLLSSEQL
KLCVLEYQGKYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNMLM
AKESLYSQLPMDCFMTPSYSRRISTATPYMNGETSTKSLWVINSALRIK
ILCATYVNVNIRDIDKIYVRTGIYHGGEPLCDNVNTQRPVCSNPRWNEW
LNYDIYIPDLPRAARLCLSI CSVKGRKGAKKEHCPLAWGNINLFDYTDI
LVSGKMALNLWVPHGLEDLLNPIGVTGSPNPKETPCLELEFDWFSSVV
KFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKA
ISTRDPLSEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQ
MYCLVKDWPPIKPEQAMELLDCNYPDPMVRGFAVRCLEKYLTDKLSQY
LIQLVQVLKYEQYLDNLLVRFLLKKALTNQRI GHFFFWHLKSEMHNKTV
SQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLTDILKQEKKDETQK
VQMKFLVEQMRRPDFMDALQGFLSPLNPAHQLGNLRLIECRIMSSAKRP
LWLNWENPDIMSELLFQNEIIFKNGDDLQDMLTLQIIRIMENIWQNQ
GLDLRMLPYGCLSIGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNSHT
LHQWLKDKNKGEIYDAAIDLFTTRSCAGYCVATFILGIGDRHNSNIMVKD
DGQLFHIDFGHFLDHKKKFGYKRERVPFVLTQDFLIVISKGAQECTKT
REFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIR
KTLALDKTEQEALEYFMKQMNDAHHGGWTTKMDWIFHTIKQHALN

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.

Division of Signal Transduction Therapy

Baculovirus
expressed
P85

MSAEGYQYRALYDYKKEREEDIDLHLGDILTVNKGSLVALGFSDGQEAR
PEEIGWLNNGYNETTGERGDFPGTYVEYIGRKKISPPTPKPRPPRPLPVA
PGSSKTEADVEQQALTLPLDLAEQFAPPDIAPPLLIKLVIAIEKKGLECS
TLYRTQSSSNLAELRQLLDCTPSVDLEMIDVHVLADAFKRYLLDLPNP
VIPAAVYSEMISLAPEVQSSEYIQLLKKLIRSPSIPHQYWLTLQYLLK
HFFKLSQTSSKNLLNARVLSEIFSPMLFRFSAASSDNTENLIKVIEILI
STEWNERQPAPALPPKPPKPTTVANNGMNNMSLQDAEWYWGDISREEV
NEKLRDTADGTFLVRDASTKMHGDYTLTLRKGGNNKLIKIFHRDGKYGF
SDPLTFSSVVELINHYRNESLAQYNPKLDVKLLYPVSKYQQDQVVKEDN
IEAVGKKLHEYNTQFOEKSREYDRLYEEYTRTSQEIOMKRTAIEAFNET
IKIFEEQCQTOERYKEYIEKFKREGNEKEIQRIMHNYDKLKSRISEII
DSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTQKG
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHHDEKTWNVGSSNRNKAENL
LRGKRDGTFLVRESSKQGCYACSVVVDGEVKHCVINKTATGYGFAEPYN
LYSSLKELVLHYQHTSLVQHNDLNVTLAYPVYAOQRR

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
GGGCATCCACTTGATGCCCCCAAGAATCCTAGTAGAATGTTTACTACC
AAATGGAATGATAGTGACTTTAGAATGCCTCCGTGAGGCTACATTAATA
ACCATAAAGCATGAACTATTTAAAGAAGCAAGAAAATACCCCTCCATC
AACTTCTTCAAGATGAATCTTCTTACATTTTTCGTAAGTGTTACTCAAGA
AGCAGAAAGGGAAGAATTTTTTGTATGAAACAAGACGACTTTGTGACCTT
CGGCTTTTTCAACCCTTTTTAAAAGTAATTGAACCAGTAGGCAACCGTG
AAGAAAAGATCCTCAATCGAGAAATTGGTTTTGTCTATCGGCATGCCAGT
GTGTGAATTTGATATGGTTAAAGATCCAGAAGTACAGGACTTCCGAAGA
AATATTCTGAACGTTTTGTAAAGAAGCTGTGGATCTTAGGGACCTCAATT
CACCTCATAGTAGAGCAATGTATGTCTATCCTCCAATGTAGAATCTTC
ACCAGAATTGCCAAAGCACATATATAATAAATTAGATAAAGGGCAAATA
ATAGTGGTGATCTGGGTAATAGTTTCTCCAATAATGACAAGCAGAAGT
ATACTCTGAAAATCAACCATGACTGTGTACCAGAACAAGTAATTGCTGA
AGCAATCAGGAAAAAACTCGAAGTATGTTGCTATCCTCTGAACAACCTA
AACTCTGTGTTTTAGAATATCAGGGCAAGTATATTTTAAAAGTGTGTG
GATGTGATGAATACTTCCTAGAAAAATATCCTCTGAGTCAGTATAAGTA
TATAAGAAGCTGTATAATGCTTGGGAGGATGCCAATTTGATGTTGATG
GCTAAAGAAAGCCTTTTATTCTCAACTGCCAATGGACTGTTTTACAATGC
CATCTTATTCCAGACGCATTTCCACAGCTACACCATATATGAATGGAGA
AACATCTACAAAATCCCTTTGGGTTATAAATAGTGCCTCAGAATAAAA
ATTCTTTGTGCAACCTACGTGAATGTAAATATTCGAGACATTGATAAGA
TCTATGTTTCGAACAGGTATCTACCATGGAGGAGAACCCTTATGTGACAA
TGTGAACACTCAAAGAGTACCTTGTCCAATCCCAGGTGGAATGAATGG
CTGAATTATGATATATACATTCCTGATCTTCCCTCGTGCTGCTCGACTTT
GCCTTTCCATTTGCTCTGTAAAGGCCGAAAGGGTGCTAAAGAGGAACA
CTGTCCATTGGCATGGGGAATATAAACTTGTGTTGATTACACAGACT
CTAGTATCTGAAAAAATGGCTTTGAATCTTTGGCCAGTACCTCATGGAT
TAGAAGATTTGCTGAACCTATTGGTGTACTGGATCAAATCCAAATAA
AGAAACTCCATGCTTAGAGTTGGAGTTTACTGTTTCCAGCAGTGTGGTA
AAGTTCCAGATATGTCAGTGATTGAAGAGCATGCCAATTGGTCTGTAT
CCCGAGAAGCAGGATTTAGCTATTTCCACGCAGGACTGAGTAACAGACT
AGCTAGAGACAATGAATTAAGGGAAAATGACAAAGAACAGCTCAAAGCA
ATTTCTACACGAGATCCTCTCTCTGAAATCACTGAGCAGGAGAAAGATT
TTCTATGGAGTCACAGACTTATTGTGTAACTATCCCCGAAATTTCTACC
CAAATTGCTTCTGTCTGTAAATGGAATTCTAGAGATGAAGTAGCCCAG
ATGTATTGCTTGGTAAAAGATTGGCCTCCAATCAAACCTGAACAGGCTA
TGGAACCTTCTGGACTGTAATTACCAGATCCTATGGTTCGAGGTTTTGC
TGTTCCGGTGCTTGGAAAAATATTTAACAGATGACAACTTTCTCAGTAT
TTAATTCAGCTAGTACAGTCCATAAATATGAACAATATTTGGATAACT
TGCTTGTGAGATTTTTACTGAAGAAAGCATTGACTAATCAAAGGATTGG
GCACTTTTTCTTTTGGCATTAAAATCTGAGATGCACAATAAAACAGTT
AGCCAGAGGTTTTGGCCTGCTTTTGGAGTCCTATTGTTCGTGCATGTGGGA
TGTATTTGAAGCACCTGAATAGGCAAGTCGAGGCAATGAAAAGCTCAT
TAACTTAACTGACATTCTCAAACAGGAGAAGAAGGATGAAACACAAAAG
GTACAGATGAAGTTTTTAGTTGAGCAAATGAGGCGACCAGATTTTCATGG
ATGCTCTACAGGGCTTTCTGTCTCCTCTAAACCTGCTCATCAACTAGG

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AAACCTCAGGCTTGAAGAGTGTCGAATTATGTCCTCTGCAAAAAGGCCA
CTGTGGTTGAATTGGGAGAACCCAGACATCATGTCAGAGTTACTGTTTC
AGAACAATGAGATCATCTTTAAAAATGGGGATGATTTACGGCAAGATAT
GCTAACACTTCAAATTATTCGTATTATGGAAAATATCTGGCAAATCAA
GGTCTTGATCTTCGAATGTTACCTTATGGTTGTCTGTCAATCGGTGACT
GTGTGGGACTTATTGAGGTGGTGCGAAATCTCACACTATTATGCAAAT
TCAGTGCAAAGGCGGCTTGAAAGGTGCACTGCAGTTCAACAGCCACACA
CTACATCAGTGGCTCAAAGACAAGAACAAAGGAGAAATATATGATGCAG
CCATTGACCTGTTTACACGTTTCATGTGCTGGATACTGTGTAGCTACCTT
CATTTTGGGAATTGGAGATCGTCACAATAGTAACATCATGGTGAAAGAC
GATGGACAACCTGTTTCATATAGATTTTGGACACTTTTTGGATCACAAGA
AGAAAAAATTTGGTTATAAACGAGAACGTGTGCCATTTGTTTTGACACA
GGATTTCTTAATAGTGATTAGTAAAGGAGCCCAAGAATGCACAAAGACA
AGAGAATTTGAGAGGTTTCAGGAGATGTGTTACAAGGCTTATCTAGCTA
TTCGACAGCATGCCAATCTCTTCATAAATCTTTTCTCAATGATGCTTGG
CTCTGGAATGCCAGAACTACAATCTTTTGATGACATTGCATACATTGCA
AAGACCCTAGCCTTAGATAAAACTGAGCAAGAGGCTTTGGAGTATTTCA
TGAAACAAATGAATGATGCACATCATGGTGGCTGGACAACAAAATGGA
TTGGATCTTCCACACAATTAACAGCATGCATTGAACTGAggtacc

Division of Signal Transduction Therapy

Complete nucleotide of sequence of P85

ATGAGTGCTGAGGGGTACCAGTACAGAGCGCTGTATGATTATAAAAAGG
AAAGAGAAGAAGATATTGACTTGCACCTGGGTGACATATTGACTGTGAA
TAAAGGGTCCTTAGTAGCTCTTGGATTCAAGTATGGACAGGAAGCCAGG
CCTGAAGAAATTGGCTGGTTAAATGGCTATAATGAAACCACAGGGGAAA
GGGGGGACTTTCCGGGAACCTACGTAGAATATATTGGAAGGAAAAAAT
CTCGCCTCCACACCAAAGCCCCGGCCACCTCGGCCTCTTCCCTGTTGCA
CCAGGTTCTTCGAAAACCTGAAGCAGATGTTGAACAACAAGCTTTGACTC
TCCCGGATCTTGCAGAGCAGTTTGCCCCCTCCTGACATTGCCCCGCCTCT
TCTTATCAAGCTCGTGGAAAGCCATTGAAAAGAAAGGTCTGGAATGTTCA
ACTCTATACAGAACACAGAGCTCCAGCAACCTGGCAGAATTACGACAGC
TTCTTGATTGTGATACACCCTCCGTGGACTTGGAAATGATCGATGTGCA
CGTTTTGGCTGACGCTTTCAAACGCTATCTCCTGGACTTACCAAATCCT
GTCATTCCAGCAGCCGTTTACAGTGAATGATTTCTTTAGCTCCAGAAG
TACAAAGCTCCGAAGAATATATTCAGCTATTGAAGAAGCTTATTAGGTC
GCCTAGCATACCTCATCAGTATTGGCTTACGCTTACAGTATTTGTTAAAA
CATTTCTTCAAGCTCTCTCAAACCTCCAGCAAAAATCTGTTGAATGCAA
GAGTACTCTCTGAAATTTTCAGCCCTATGCTTTTCAGATTCTCAGCAGC
CAGCTCTGATAATACTGAAAACCTCATAAAAGTTATAGAAATTTTAATC
TCAACTGAATGGAATGAACGACAGCCTGCACCAGCACTGCCTCCTAAAC
CACAAAACCTACTACTGTAGCCAACAACGGTATGAATAACAATATGTC
CTTACAAGATGCTGAATGGTACTGGGGAGATATCTCGAGGGAAGAAGTG
AATGAAAACTTCGAGATACAGCAGACGGGACCTTTTTTGGTACGAGATG
CGTCTACTAAAATGCATGGTGATTATACTCTTACACTAAGGAAAGGGG
AAATAACAAATTAATCAAAATATTTTCATCGAGATGGGAAATATGGCTTC
TCTGACCCATTAACCTTCAGTTCTGTGGTTGAATTAATAAACCACTACC
GGAATGAATCTCTAGCTCAGTATAATCCCAAATGGATGTGAAATTACT
TTATCCAGTATCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAAT
ATTGAAGCTGTAGGGAAAAAATTACATGAATATAACACTCAGTTTCAAG
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CCAGGAAATCCAAATGAAAAGGACAGCTATTGAAGCATTTAATGAAACC
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AATACATAGAAAAGTTTAAACGTGAAGGCAATGAGAAAGAAATACAAAG
GATTATGCATAATTATGATAAGTTGAAGTCTCGAATCAGTGAAATTATT
GACAGTAGAAGAAGATTGGAAGAAGACTTGAAGAAGCAGGCAGCTGAGT
ATCGAGAAATTGACAAACGTATGAACAGCATTAAACCAGACCTTATCCA
GCTGAGAAAGACGAGAGACCAATACTTGTATGTGGTTGACTCAAAAAGGT
GTTTCGGCAAAGAAGTTGAACGAGTGGTTGGGCAATGAAAACACTGAAG
ACCAATATTCCTGTTGGAAGATGATGAAGATTTGCCCATCATGATGA
GAAGACATGGAATGTTGGAAGCAGCAACCGAAACAAAGCTGAAAACCTG
TTGCGAGGGAAGCGAGATGGCACTTTTCTTGTCCGGGAGAGCAGTAAAC
AGGGCTGCTATGCCTGCTCTGTAGTGGTGGACGGCGAAGTAAAGCATTG
TGTCATAAACAAAACAGCAACTGGCTATGGCTTTGCCGAGCCCTATAAC
TTGTACAGCTCTCTGAAAGAACTGGTGCTACATTACCAACACACCTCCC
TTGTGCAGCACAACGACTCCCTCAATGTCACACTAGCCTACCCAGTATA
TGCACAGCAGAGGCGatgaactagt