

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PI3 kinase alpha E545K [2 – 1068] / p85 [1 - 724]

<u>Enzyme description:-</u>	PI3 kinase alpha E542K [2 – 1068] / p85 [1 - 724]
<u>Clone number:-</u>	DU 12812
<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, 024.43 daltons [PI3kinase alpha] and 83, 546.54 daltons [P85]
Average Mass	125, 106.00 daltons [PI3kinase alpha] and 83, 598.39 daltons [P85]
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.19 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 E545K [2 – 1068] / p85 [1 - 724]

Protein PI3 kinase alpha E545K [2 – 1068] / p85 [1 – 724]

Clone number DU 12812

Species Human

Accession number PI3 kinase alpha U79143 / p85 NM_181523

Tags N-terminal His(6)

Baculovirus expressed
PI3 kinase alpha

MHHHHHHPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLI
TIKHELFKEARKYPLHQLLQDESSYIFVSVTQEAEREFFDETTRLCDL
RLFQPFLLKVIIEPVGNREEKILNREIGFAIGMPVCEFDLVKDEVDQDFRR
NILNVCKEAVDLRLNSPHSRAMYVYPPNVESSELPKHIYNKLDKGQI
IVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIKKKTRSMLLSSEQL
KLCVLEYQGGYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNMLM
AKESLYSQLPMDCFMTSPYSRRISTATPYMNGETSTKSLWVINSALRIK
ILCATYVNVNIRDIDKIYVRTGIYHGGEPLCDNVNTQRPVPCSNPRWNEW
LNYDIYIPDLPRARLCLCSVKGKRGAKKEHCPLAWGNINLFDYTDI
LVSGKMALNLWVPVPHGLEDLLNPIGVTGSNPNKETPCLELEFDWFSSVV
KFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKA
ISTRDPLSEITKQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQ
MYCLVKDWPPIKPEQAMELLDCNYPDPMVRGFAVRCLEKYLTDDKLSQY
LIQLVQVLKYEQYLDNLLVRFLKALTNQRIGHFFFWHLKSEMHNKT
SQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLTDILKQEKKDETQK
VQMKFLVEQMRRPDFMDALQGFLSPLNPAHQGLNLRLEECRIMSSAKRP
LWLNWENPDIMSELLFQNNIEIFKNGDDLQDMLTLQIIRIMENIWQNO
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 enzyme has an E545K mutation to mimic the activation mutation found in many types of cancers. Residue E545 is equivalent to K551 of the fusion protein. The His(6) tag is located at residues 2 – 7.

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Baculovirus expressed P85

MSAEGYQYRALYDYKKEREEDIDLHLGDILTVNKGSLVALGFSDGQEAR
PEEIGWLNGYNETTGERGDFPGTYVEYIGRKKISPPTPKPRPPRPLPVA
PGSSKTEADVEQQALTLPDLAEQFAPPDIAPELLIKLVEAIEKKGLECS
TLYRTQSSSNLAELRQLLDCTPSVDLEMIDVHVLADAFKRYLLDLPNP
VIPAAVYSEMISLAPEVQSSEEIYQLLKKLIRSPSIPHQYWLTLQYLLK
HFFKLSQTSSKNLLNARVLSEIFSPMLFRFSAASSDNTENLIKVIEILI
STEWNERQPAPALPPKPPKPTTVANNGMNNNMSLQDAEWYWGDISREEV
NEKLRTADGTFLVRDASTKMHGDYTLTLRKGGNNKLIKIFHRDGKYGF
SDPLTFSSVVELINHYRNESLAQYNPKLDVKLLYPVSKYQQDQVVKEDN
IEAVGKKLHEYNTQFQEKsREYDRLYEEYTRTSQEIQMKRTAIEAFNET
IKIFEEQCQTQERYsKEYIEKFKREGNEKEIQRIMHNYDKLKSRISEII
DSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTQKG
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHHDEKTWNVGSSNRNKAENL
LRGKRDGTFLVRESSKQGCYACSVVVDGEVKHCVINKTATGYGFAEPYN
LYSSLKELVLHYQHTSLVQHNDSLNVTLAYPVYAQQRR

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
AAGAAAAGATCCTCAATCGAGAAATTGGTTTTTGTATCGGCATGCCAGT
GTGTGAATTTGATATGGTTAAAGATCCAGAAGTACAGGACTTCCGAAGA
AATATTCTGAACGTTTTGTAAAGAAGCTGTGGATCTTAGGGACCTCAATT
CACCTCATAGTAGAGCAATGTATGTCTATCCTCCAATGTAGAATCTTC
ACCAGAATTGCCAAAGCACATATATAATAAATTAGATAAAGGGCAAATA
ATAGTGGTGATCTGGGTAATAGTTTCTCCAAATAATGACAAGCAGAAGT
ATACTCTGAAAATCAACCATGACTGTGTACCAGAACAAGTAATTGCTGA
AGCAATCAGGAAAAAACTCGAAGTATGTTGCTATCCTCTGAACAACCTA
AACTCTGTGTTTTAGAAATATCAGGGCAAGTATATTTTAAAAGTGTGTG
GATGTGATGAATACTTCCTAGAAAAATATCCTCTGAGTCAGTATAAGTA
TATAAGAAGCTGTATAATGCTTGGGAGGATGCCAATTTGATGTTGATG
GCTAAAGAAAGCCTTTATTCTCAACTGCCAATGGACTGTTTTACAATGC
CATCTTATTCCAGACGCATTTCCACAGCTACACCATATATGAATGGAGA
AACATCTACAAAATCCCTTTGGGTTATAAATAGTGCACTCAGAATAAAA
ATTCTTTGTGCAACCTACGTGAATGTAAATATTTCGAGACATTGATAAGA
TCTATGTTTCGAACAGGTATCTACCATGGAGGAGAACCCTTATGTGACAA
TGTGAACACTCAAAGAGTACCTTGTTCCAATCCCAGGTGGAATGAATGG
CTGAATTATGATATATACATTCCCTGATCTTCCTCGTGCTGCTCGACTTT
GCCTTTCCATTTGCTCTGTAAAGGCCGAAAGGGTGCTAAAGAGGAACA
CTGTCCATTGGCATGGGGAAATATAAACTTGTTTGATTACACAGACACT
CTAGTATCTGGAAAAATGGCTTTGAATCTTTGGCCAGTACCTCATGGAT
TAGAAGATTTGCTGAACCTATTGGTGTTACTGGATCAAATCCAAATAA
AGAACTCCATGCTTAGAGTTGGAGTTTGAAGTTTCTGAGTGTGGTA
AAGTTCCAGATATGTCAGTGATTGAAGAGCATGCCAATTGGTCTGTAT
CCCGAGAAGCAGGATTTAGCTATTCCACGCAGGACTGAGTAACAGACT
AGCTAGAGACAATGAATTAAGGGAAAATGACAAAGAACAGCTCAAAGCA
ATTTCTACACGAGATCCTCTCTCTGAAATCACTAAGCAGGAGAAAGATT
TTCTATGGAGTCACAGACACTATTGTGTAAGTATCCCCGAAATCTACC
CAAATTGCTTCTGTCTGTAAATGGAATTCTAGAGATGAAGTAGCCCAG
ATGTATTGCTTGGTAAAAGATTGGCCTCCAATCAAACCTGAACAGGCTA
TGGAACCTTCTGGACTGTAATTACCCAGATCCTATGGTTTCGAGGTTTTGC
TGTTCCGGTGCTTGGAAAAATATTTAACAGATGACAACTTTCTCAGTAT
TTAATTCAGCTAGTACAGGTCCTAAATATGAACAATATTTGGATAACT
TGCTTGTGAGATTTTTTACTGAAGAAAGCATTGACTAATCAAAGGATTGG
GCACTTTTTCTTTTGGCATTATAAATCTGAGATGCACAATAAAACAGTT
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
TAAAGGGTCCTTAGTAGCTCTTGGATTCAGTGATGGACAGGAAGCCAGG
CCTGAAGAAATTGGCTGGTTAAATGGCTATAATGAAACCACAGGGGAAA
GGGGGGACTTTCCGGGAACTTACGTAGAATATATTGGAAGGAAAAAAT
CTCGCCTCCCACACCAAAGCCCCGGCCACCTCGGCCTCTTCCTGTTGCA
CCAGGTTCTTCGAAAACCTGAAGCAGATGTTGAACAACAAGCTTTGACTC
TCCCGGATCTTGCAGAGCAGTTTGCCCCCTCCTGACATTGCCCCGCCTCT
TCTTATCAAGCTCGTGGAAGCCATTGAAAAGAAAGGTCTGGAATGTTCA
ACTCTATACAGAACACAGAGCTCCAGCAACCTGGCAGAATTACGACAGC
TTCTTGATTGTGATACACCCTCCGTGGACTTGGAATGATCGATGTGCA
CGTTTTGGCTGACGCTTTCAAACGCTATCTCCTGGACTTACCAAATCCT
GTCATTCCAGCAGCCGTTTACAGTGAAATGATTTCTTTAGCTCCAGAAG
TACAAAGCTCCGAAGAATATATTCAGCTATTGAAGAAGCTTATTAGGTC
GCCTAGCATACCTCATCAGTATTGGCTTACGCTTCAGTATTTGTTAAAA
CATTTCTTCAAGCTCTCTCAAACCTCCAGCAAAAATCTGTTGAATGCAA
GAGTACTCTCTGAAATTTTTCAGCCCTATGCTTTTTCAGATTCTCAGCAGC
CAGCTCTGATAATACTGAAAACCTCATAAAAGTTATAGAAATTTTAATC
TCAACTGAATGGAATGAACGACAGCCTGCACCAGCACTGCCTCCTAAAC
CACCAAACCTACTACTGTAGCCAACAACGGTATGAATAACAATATGTC
CTTACAAGATGCTGAATGGTACTGGGGAGATATCTCGAGGGAAGAAGTG
AATGAAAACTTCGAGATACAGCAGACGGGACCTTTTTTGGTACGAGATG
CGTCTACTAAAATGCATGGTGATTATACTCTTACACTAAGGAAAGGGG
AAATAACAAATTAATCAAAATATTTTCATCGAGATGGGAAATATGGCTTC
TCTGACCCATTAACCTTCAGTTCTGTGGTTGAATTAATAAACCACTACC
GGAATGAATCTCTAGCTCAGTATAATCCCAAATTGGATGTGAAATTACT
TTATCCAGTATCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAAT
ATTGAAGCTGTAGGGAAAAAATTACATGAATATAACACTCAGTTTCAAG
AAAAAAGTCGAGAATATGATAGATTATATGAAGAATATACCCGCACATC
CCAGGAAATCCAAATGAAAAGGACAGCTATTGAAGCATTTAATGAAACC
ATAAAAATATTTGAAGAACAGTGCCAGACCCAAGAGCGGTACAGCAAAG
AATACATAGAAAAGTTTAAACGTGAAGGCAATGAGAAAGAAATACAAAG
GATTATGCATAATTATGATAAGTTGAAGTCTCGAATCAGTGAAATTATT
GACAGTAGAAGAAGATTGGAAGAAGACTTGAAGAAGCAGGCAGCTGAGT
ATCGAGAAATTGACAAACGTATGAACAGCATTAACCAGACCTTATCCA
GCTGAGAAAGACGAGAGACCAATACTTGATGTGGTTGACTCAAAAAGGT
GTTTCGGCAAAAGAAGTTGAACGAGTGGTTGGGCAATGAAAACACTGAAG
ACCAATATTTCACTGGTGGAAGATGATGAAGATTTGCCCATCATGATGA
GAAGACATGGAATGTTGGAAGCAGCAACCGAAACAAAGCTGAAAACCTG
TTGCGAGGGAAGCGAGATGGCACTTTTCTTGTCCGGGAGAGCAGTAAAC
AGGGCTGCTATGCCTGCTCTGTAGTGGTGGACGGCGAAGTAAAGCATTG
TGTCATAAACAAAACAGCAACTGGCTATGGCTTTGCCGAGCCCTATAAC
TTGTACAGCTCTCTGAAAGAACTGGTGCTACATTACCAACACACCTCCC
TTGTGCAGCACAACGACTCCCTCAATGTCACACTAGCCTACCCAGTATA
TGCACAGCAGAGGCGatgaactagt