

# *Division of Signal Transduction Therapy*

## **Standard Operating Procedure**

### **Preparation of active PI3 kinase alpha E542K [2 – 1068] / p85 [1 - 724]**

**Enzyme description:-** PI3 kinase alpha E542K [2 – 1068] / p85 [1 - 724]

**Clone number:-** DU 12756

**Source:-** Recombinant

**Expression system:-** Baculovirus expression vector system

**Tag:-** N-terminal His(6) PI3 kinase alpha  
No tag for p85

**Purification method:-** Ni<sup>2+</sup>-NTA agarose

**Expression level:-** 3 mg/L

#### **Calculated molecular mass:-**

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]

**Theoretical pI:-** 7.19 for PI3kinase alpha and 5.84 for P85

**Purity:-** >80 %

**Activation protocol:-** Constitutively active

#### **Enzyme storage buffer:-**

50mM Hepes/NaOH pH7.0, 150 mM NaCl, 5 mM DTT, 20 % glycerol

**Storage temperature:-** -70 °C

**Assay:-** ADP Glo

#### **Assay Buffer:-**

12.5 mM glycine-NaOH pH 8.5, 50 mM KCl, 1 mM DTT, 0.05 % CHAPS, 2.5 mM MgCl<sub>2</sub>

#### **Substrate:-**

PI (4,5)P<sub>2</sub> diC8            Final concentration: 0.05 mM

# *Division of Signal Transduction Therapy*

## Clone Data Sheet

### PI3 kinase alpha E542K [2 – 1068] / p85 [1 - 724]

<u>Protein</u>	PI3 kinase alpha [2 – 1068] / p85 [1 – 724]
<u>Clone number</u>	DU 12756
<u>Species</u>	Human
<u>Accession number</u>	PI3 kinase alpha U79143 / p85 NM_181523
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed PI3 kinase alpha</u>	MHHHHHHPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLI TIKH <del>E</del> LFKEARKYPLH <del>H</del> QLLQDESSYIFVSVTQEAEEREEFFDET <del>R</del> RLCDL RLFQPFLK <del>V</del> IEPVGNREEKILNREIGFAIGMPVCEFDMVKDPEVQDFRR NILNVCKEAVDLRDLNSPHSRAMYVYPPNVESSPELPKHIYNKLDKGQI IVVIWIVIVSPNNDKQKYTLKINHDCVPEQVIAEAIRKKTRSM <del>L</del> SSEQL KLCVLEYQGKYILKVC <del>G</del> CDEYFLEKYPLSQYKYIRSCIMLGRMPNLMLM AKESLYSQLPMDCFTMPSYSRRISTATPYMNGETSTKSLWINSALRIK ILCATYVN <del>V</del> NIRDIDKIYVRTGIYHGGEPLCDNVNTQRVPCSNPRWNEW LNYDIYIPDLPRAARLCLSICSVKGRKGAKEEHCPLAWGNINLFDYTDT LVSGK <del>M</del> ANLWPVPVHGLEDLLNPIGVTGSNPNKETPCLEFDFWSSVV KFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKA ISTRDPLSKITEQEKDFLWSHRHYCVTIPEILPK <del>L</del> LSVKWNSRDEVAQ MYCLVKDW <del>P</del> PIKPEQAM <del>E</del> LLDCNYPDPMVRGFAVRCLEKYLTDDKLSQY LIQLVQLKYEQYLDNLLVRF <del>L</del> KKALT <del>N</del> Q <del>R</del> I <del>G</del> FFF <del>W</del> HLKSEMHNKT <del>V</del> SQRFG <del>L</del> LESYC <del>R</del> ACGMYLKHLNRQ <del>V</del> EAMEKL <del>I</del> NLTD <del>I</del> LKQEK <del>K</del> DET <del>Q</del> VQMKFLVEQMRRPDFMDALQGFLSPLNPAHQLGNLR <del>L</del> EECRIMSSAKRP LWLNWENPDIMSELLFQNNEIIFKNGDDL <del>R</del> QDM <del>L</del> T <del>L</del> QIIRIMENIWQ <del>Q</del> GLDLRMLPYG <del>C</del> L <del>S</del> IGDCVGLIEVVRNSHTIM <del>O</del> IQCKGG <del>L</del> KGALQFN <del>S</del> H <del>T</del> LHQWLKDKNKGEIYDAAI <del>D</del> L <del>F</del> TRSCAGYC <del>V</del> ATF <del>I</del> LGIGDRHNSNIMV <del>K</del> DGQLFHIDFGHFLDHKKKKFGYKRERV <del>P</del> VLTQDFLIVISKGAQECT <del>K</del> REFERFOEMCYKAYLAIRQHANLF <del>I</del> NLF <del>S</del> MLGSGMPELQS <del>F</del> DDIAYIR KTLALDKTEQEALEYFMKQMNDAHGGWTTKMDWIFHTIKQHALN
<u>Native sequence PI3 kinase alpha</u>	Amino acids P2 – N1068 (end) of human PI3 kinase alpha. Residue P8 of the fusion protein is equivalent to P2 of the native enzyme. The enzyme has a E542K mutation to mimic the activation mutation found in many types of cancers. Residues E542 is equivalent to K548 of the fusion protein. The His(6) tag is located at residues 2 – 7.

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Baculovirus  
expressed  
P85

MSAEGYQYRALYDYKKEREEDIDLHLDILTVNKGSIVALGFSDGQEAR  
PEEIGWLNGYNETTGERGDFPGTYVEYIGRKKISPPTPKPRPPRPLPVA  
PGSSKTEADVEQQALTLPLAEQFAPPDIAPPLLIKLVIAIEKKGLECS  
TLYRTQSSNLAELRQLLDCDTPSVDEIMDVHLADAFKRYLLDLPNP  
VIPAAYVSEMIISLAPEVQSSEEEYIQLLKKLIRSPSIPHQYWLTQYLLK  
HFFKLSQTSSKNLLNARVLSEIFSPMLFRFSAAASSDNTENLIKVIEILI  
STEWNERQPAPALPPKPPKPTTVANNGMNNNMSLQDAEWYWGDISREEV  
NEKLRDTADGTFLVRDASTKMHGDYTLTLRKGGNNKLIKIFHRDGKYGF  
SDPLTFSSVVELINHYRNESLAQYNPKLDVKLLYPVSKYQDQVVKEDN  
IEAVGKKLHEYNTQFQEKSREYDRLYEYRTSQEIQMRTAIEAFNET  
IKIFEEQCQTQERYSKKEYIEKFREGNEKEIQRIMHNYDKLKSRISEII  
DSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTKKG  
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHDEKTWNVGSSRNKAENL  
LRGKRDGTFLVRESSKQGCYACSVVDGEVKHCVINKTATGYGFAEPYN  
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  
GGGGCATCCACTGATGCCCAAGAACATCCTAGTAGAATGTTACTACC  
AAATGGAATGATAGTGACTTTAGAATGCCTCGTGAGGCTACATTATA  
ACCATAAAGCATGAACTATTTAAAGAACAGCAAGAAAATACCCCTCCATC  
AACTTCTCAAGATGAATCTTCTACATTTCTGAAGTGTACTCAAGA  
AGCAGAAAGGGAGAACATTGGATGAAACAAGACGACTTGTGACCTT  
CGGCTTTCAACCCTTTAAAAGTAATTGAACCAGTAGGCAACCGTG  
AAGAAAAGATCCTCAATCGAGAACATTGGTTGCTATCGGCATGCCAGT  
GTGTGAATTGATATGGTTAAAGATCCAGAAGTACAGGACTTCCGAAGA  
AATATTCTGAACGTTGTAAAGAACGCTGTGATCTTAGGGACCTCAATT  
CACCTCATAGTAGAGCAATGTATGTCTATCCTCAAATGTAGAATCTTC  
ACCAGAATTGCCAAAGCACATATATAATAAAATTAGATAAAGGGCAAATA  
ATAGTGGTGTCTGGTAATAGTTCTCAAATAATGACAAGCAGAAGT  
ATACTCTGAAAATCAACCATGACTGTGTACAGAACAGTAATTGCTGA  
AGCAATCAGGAAAAAAACTCGAAGTATGTTGCTATCCTCTGAACAACTA  
AAACTCTGTGTTTAGAATATCAGGGCAAGTATATTAAAAGTGTGTG  
GATGTGATGAATACTCCTAGAAAATATCCTCTGAGTCAGTATAAGTA  
TATAAGAAGCTGTATAATGCTTGGGAGGATGCCAATTGATGTTGATG  
GCTAAAGAAAGCCTTATTCTCAACTGCCAATGGACTGTTACAATGC  
CATCTTATTCCAGACGCATTCCACAGCTACACCATAATGAATGGAGA  
AACATCTACAAAATCCCTTGGTTATAAATAGTCAGAACAGAAATAAA  
ATTCTTGTGCAACCTACGTGAATGAAATATTGAGACATTGATAAGA  
TCTATGTCGAACAGGTATCTACCATGGAGGAGAACCTTATGTGACAA  
TGTGAACACTCAAAGAGTACCTGTTCCAATCCCAGGTGGAATGAATGG  
CTGAATTATGATATATACATTCTGATCTCCTCGTGTGCTCGACTTT  
GCCTTCATTGCTCTGTTAAAGGCCAAAGGGTCTAAAGAGGAACA  
CTGTCCATTGGCATGGGAAATATAAACTGTTGATTACACAGACACT  
CTAGTATCTGAAAAATGGCTTGAATCTTGGCCAGTACCTCATGGAT  
TAGAAGATTGCTGAACCTATTGGTGTACTGGATCAAATCAAATAA  
AGAAACTCCATGCTTAGAGTTGGAGTTGACTGGTTCAGCAGTGTGGTA  
AAGTCCCAGATATGTCAGTGAAGAGCATGCCAATTGGCTGTAT  
CCCGAGAAGCAGGATTAGCTATTCCACGCAGGACTGAGTAACAGACT  
AGCTAGAGACAATGAATTAAGGAAAATGACAAAGAACAGCTCAAAGCA  
ATTCTACACGAGATCCTCTCTAAATCACTGAGCAGGAGAAAGATT  
TTCTATGGAGTCACAGACACTATTGTGTAACTATCCCCGAAATTCTACC  
CAAATTGCTCTGCTGTTAAATGAAATTCTAGAGATGAAGTAGCCCAG  
ATGTATTGCTGGTAAAGATTGGCTCCAATCAAACCTGAACAGGCTA  
TGGAACCTCTGGACTGTAATTACCCAGATCCTATGGTCAGGTTTGC  
TGTCGGCTGGAAAAATATTAAACAGATGACAAACTTCTCAGTAT  
TTAATTCACTAGTACAGGCTCTAAATATGAACAATATTGGATAACT  
TGCTTGTGAGATTGACTGAAAGAACAGTCAATCAAAGGATTGG  
GCACTTTCTGGCATTAAATCTGAGATGCACAATAAACAGTT  
AGCCAGAGGTTGGCCTGCTTGGAGTCCTATTGTCGTGCATGTGGGA  
TGTATTGAAAGCACCTGAATAGGCAAGTCGAGGCAATGGAAAAGCTCAT  
TAACTTAACTGACATTCTAAACAGGAGAACAGGATGAAACACAAAAG  
GTACAGATGAAGTTTAGTTGAGCAAATGAGGCGACCAGATTCATGG  
ATGCTCTACAGGGCTTCTGTCCTCTAAACCCTGCTCATCAACTAGG

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AAACCTCAGGCTTGAAGAGTGTCAATTATGTCCTCTGCAAAAAGGCCA  
CTGTGGTTGAATTGGGAGAACCCAGACATCATGTCAGAGTTACTGTTTC  
AGAACAAATGAGATCATCTTAAAAATGGGGATGATTACGGCAAGATA  
GCTAACACTCAAATTATTCGTATTATGGAAAATATCTGGCAAATCAA  
GGTCTTGATCTCGAATGTTACCTTATGGTTGTCTGCAATCGGTGACT  
GTGTGGGACTTATTGAGGGTGGTGCAGAAATTCTCACACTATTATGCAAAT  
TCAGTGCAAAGGCGGCTTGAAAGGTGCACTGCAGTTAACAGGCCACACA  
CTACATCAGTGGCTCAAAGACAAGAACAAAGGAGAAATATATGATGCAG  
CCATTGACCTGTTACACGTTACATGTCAGTGGATACTGTGTAGCTACCTT  
CATTGGAAATTGGAGATCGTCACAATAGTAACATCATGGTGAAGAC  
GATGGACAACGTGTTCATATAGATTGGACACTTTGGATCACAAGA  
AGAAAAAAATTGGTTATAAACGAGAACGTGTGCCATTGTTGACACA  
GGATTCTTAATAGTAGTAAAGGAGCCAAGAACATGCACAAAGACA  
AGAGAATTGAGAGGTTAGGAGATGTGTTACAAGGCTTGGAGTATTCA  
TTCGACAGCATGCCAATCTTCATAAATCTTCTCAATGATGCTTGG  
CTCTGGAATGCCAGAACTACAATCTTGATGACATTGCATACATTCA  
AAGACCCTAGCCTAGATAAAACTGAGCAAGAGGCTTGGAGTATTCA  
TGAAACAAATGAATGATGCACATCATGGTGGCTGGACAACAAAAATGGA  
TTGGATCTTCCACACAATTAAACAGCATGCAATTGAACTgaggtaacc

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**Complete  
nucleotide  
of sequence of P85**

ATGAGTGCTGAGGGGTACCACTACAGAGCGCTGTATGATTATAAAAAGG  
AAAGAGAAGAAGATATTGACTTGCACCTGGGTGACATATTGACTGTGAA  
TAAAGGGCCTTAGCTCTGGATTCACTGATGGACAGGAAGCCAGG  
CCTGAAGAAATTGGCTGGTTAAATGGCTATAATGAAACCACAGGGAAA  
GGGGGGACTTCCGGAACTTACGTAGAATATATTGGAAGGAAAAAAAT  
CTCGCCTCCCACACCAAAGCCCCGCCACCTCGGCCTTCCTGTTGCA  
CCAGGTTCTCGAAAACCTGAAGCAGATGTTGAACAACAAGCTTGACTC  
TCCCAGATCTGAGCAGAGCAGTTGCCCTCCTGACATTGCCCGCTCT  
TCTTATCAAGCTCGTGGAGGCCATTGAAAAGAAAGGTCTGGAATGTTCA  
ACTCTATACAGAACACAGAGCTCCAGCAACCTGGCAGAATTACGACAGC  
TTCTTGATTGTGATACACCCCTCGTGGACTTGGAAATGATCGATGTGCA  
CGTTTGGCTGACGCTTCAAACGCTATCTCCTGGACTTACCAAATCCT  
GTCATTCCAGCAGCCGTTACAGTGAATGATTCTTAGCTCCAGAAG  
TACAAAGCTCCGAAGAATATATTAGCTATTGAAGAAGCTTATTAGGT  
GCCTAGCATACTCATCAGTATTGGCTTACGCTTACAGTATTGTTAAAA  
CATTCTCAAGCTCTCAAACCTCCAGCAAAATCTGTTGAATGCAA  
GAGTACTCTGAAATTTCAGCCATGCTTTAGATTCTCAGCAG  
CAGCTCTGATAATACTGAAAACCTCATAAAAGTTATAGAAATTAACT  
TCAACTGAATGGAATGAACGACAGCCTGCACCAGCACTGCCTCTAAAC  
CACCAAAACCTACTACTGTAGCCAACAACGGTATGAATAACAATATGTC  
CTTACAAGATGCTGAATGGTACTGGGAGATATCTGAGGGAAAGAAGTG  
AATGAAAAACTTCGAGATACAGCAGACGGGACCTTTGGTACGAGATG  
CGTCTACTAAAATGCATGGTATTACTCTACACTAAGGAAAGGGGG  
AAATAACAAATTAATCAAATATTTCATCGAGATGGAAATATGGCTTC  
TCTGACCCATTAAACCTCAGTTCTGTTGAATTAAACCAACTACC  
GGAATGAATCTCTAGCTCAGTATAATCCAAATTGGATGTGAAATTACT  
TTATCCAGTATCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAAT  
ATTGAAGCTGTAGGGAAAAAATTACATGAATATAACACTCAGTTCAAG  
AAAAAAGTCGAGAATATGATAGATTATGAAGAATATAACCCGCACATC  
CCAGGAAATCAAATGAAAAGGACAGCTATTGAAGCATTAAATGAAACC  
ATAAAATATTGAAGAACAGTGCCAGACCAAGAGCGGTACAGCAAAG  
AATACATAGAAAAGTTAACGTGAAGGCAATGAGAAAGAAATACAAAG  
GATTATGCATAATTATGATAAGTTGAAGTCTGAATCAGTGAATTATT  
GACAGTAGAAGAAGATTGGAAGAAGACTTGAAGAAGCAGGCAGCTGAGT  
ATCGAGAAATTGACAAACGTATGAACAGCATTAAACCAAGACCTTATCCA  
GCTGAGAAAGACGAGAGACCAATACTTGATGTGGTTGACTCAAAAGGT  
GTTCGGCAAAAGAAGTTGAACGAGTGGTTGGCAATGAAAACACTGAAG  
ACCAATATTCACTGGTGGAAAGATGATGAAGATTGCCCCATCATGATGA  
GAAGACATGGAATGTTGGAAGCAGCAACCGAAACAAAGCTGAAAACCTG  
TTGCGAGGGAAAGCGAGATGGCACTTTCTGTCCGGGAGAGCAGTAAAC  
AGGGCTGCTATGCCTGCTGTAGTGGTGGACGGCGAAGTAAAGCATTG  
TGTCTAAACAAACAGCAACTGGCTATGGCTTGGCGAGCCCTATAAC  
TTGTACAGCTCTGAAAGAACTGGTGTACATTACCAACACACCTCCC  
TTGTGCAGCACAAAGACTCCCTCAATGTCACACTAGCCTACCCAGTATA  
TGCACAGCAGAGGCGAtgaactagt