

## Certificate of Analysis

### PI 3-Kinase (p110 $\alpha$ (E542K)/p85 $\alpha$ )

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-782, 14-782-K, 14-782M

Parent Lot # 1627191

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** Complex of *N*-terminal 6His-tagged, recombinant, full-length human p110 $\alpha$  containing the mutation E542K and untagged, recombinant, full-length human p85 $\alpha$ . Co-expressed by baculovirus in Sf21 insect cells and purified using Ni<sup>2+</sup>/NTA-agarose.

The E542K substitution is a somatic mutation in p110 $\alpha$  that has been associated with tumours of the colon and brain. Combined *in vitro* and *in vivo* studies have shown that this mutation confers higher lipid kinase activity than wild type, and is able to induce oncogenic transformation. (Kang S. *et al.*, PNAS, (2005);**102**: 802-807 and Zhao J.J. *et al.*, PNAS, (2005);**102**:18443-18448).

Purity (p110 $\alpha$  & p85 $\alpha$  combined) 93.9% by SDS-PAGE and Coomassie blue staining. p110 $\alpha$ (E542K) MW = 125.3kDa, p85 $\alpha$  MW = 83.7kDa.

**Specific Activity (Parent lot# 1627191):** 398U/mg, where one unit of PI 3-Kinase alpha (p110 $\alpha$ (E542K)/p85 $\alpha$  (h)) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate (PIP3) formed per minute at room temperature with a final ATP concentration of 100 $\mu$ M.

**Formulation:** 1.115mg/ml of enzyme in 50mM Tris/HCl pH8.0, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

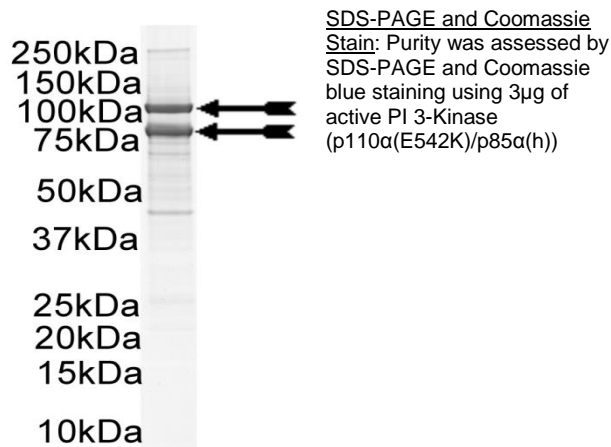
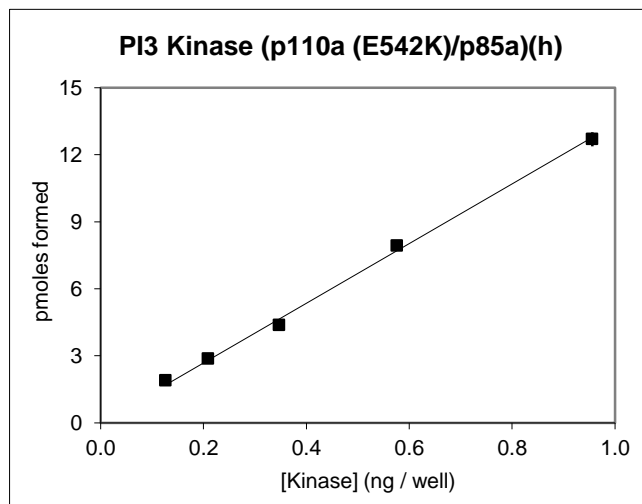
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 0.1–0.98ng of this enzyme phosphorylated 10 $\mu$ M phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.

**MS Tryptic Fingerprint:** Confirmed product identity as PI 3-Kinase (p110 $\alpha$ /p85 $\alpha$ ) with the p110 $\alpha$  and p85 $\alpha$  translated sequence listed on pages three and five.



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### Eurofins PI3 Kinase Homogeneous Time-resolved Fluorescence (HTRF) Class I Reagent Kits

The following Eurofins kits are suitable for use with this enzyme:

Cat. No	Kit Description
33-016	PI3 Kinase 4-Step Assay Reagent 1-Plate Kit
33-017	PI3 Kinase 4-Step Assay Reagent 5-Plate Kit
33-036	PI3 Kinase 4-Step Assay Reagent Kit (10000 wells)
33-037	PI3 Kinase 4-Step Assay Reagent Kit (50000 wells)
33-040	PI3 Kinase 3-Step Assay Reagent Kit (384 wells)
33-041	PI3 Kinase 3-Step Assay Reagent Kit (1920 wells)
33-047	PI3 Kinase 3-Step Assay Reagent Kit (10000 wells)

Kits 33-016, 33-017, 33-036 and 33-037 provide reagents and assay details for the Eurofins standard 4-step HTRF assay. This assay format is suitable for the majority of small and medium throughput screening work. The 3-step HTRF assay (kits 33-040, 33-041, 33-047) was introduced to reduce the number of assay steps to aid high throughput screening. Items 33-040 and 33-041 are intended as introductory kits for 3-step procedure work up. Please contact us for any further information regarding different kit formats ([discoveryservices@eurofins.com](mailto:discoveryservices@eurofins.com)).

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### p110 $\alpha$ (E542K) Sequence Information

<b><u>Protein</u></b>	Human p110 $\alpha$ (E542K)
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	M8 of the recombinant protein is equivalent to M1 of human p110 $\alpha$
<b><u>Accession number</u></b>	GenBank U79143

#### Recombinant p110 $\alpha$ (E542K) amino acid sequence:

```

1  MHHHHHHMPP  RPSSGELWGI  HLMPPRILVE  CLLPNGMIVT  LECLREATLI  TIKHELKFEA
61  RKYPLHQLLQ  DESSYIFVSV  TQEAEREFF  DETRRLCDLR  LFPQFLKVIE  PVGNREEKIL
121 NREIGFAIGM  PVCEFDMVKD  PEVQDFRNI  LNVCKEAVDL  RDLNSPHSRA  MYVYPPNVES
181 SPELPKHIYN  KLDKGQIIIV  IWVIVSPNND  KQKYTLKINH  DCVPEQVIAE  AIRKKTRSML
241 LSSEQLKLCV  LEYQGKYILK  VCGCDEYFLE  KYPLSQYKYI  RSCIMLGRMP  NLMLMAKESL
301 YSQLPMDCF  MPSYSRRIST  ATPYMNGETS  TKSLWVINS  LRIKILCATY  VNVNIRDIDK
361 IYVRTGIYHG  GEPLCDNVNT  QRVPCSNPRW  NEWLNYDIYI  PDLPRAARLC  LSICSVKGRK
421 GAKEEHCPLA  WGNINLFDYT  DTLVSGKMAL  NLWPVPHGLE  DLLNPIGVTG  SNPNKETPCL
481 ELEFDWFSSV  VKFPDMSVIE  EHANWSVSRE  AGFSYSHAGL  SNRLARDNEL  RENDKEQLKA
541 ISTRDPLSKI  TEQEKDFLWS  HRHYCVTIPE  ILPKLLLSVK  WNSRDEVAQM  YCLVKDWPPI
601 KPEQAMELLD  CNYPDPMVRG  FAVRCLEKYL  TDDKLSQYLI  QLVQVLKYEQ  YLDNLLVRF
661 LKKALTNQRI  GHFFFWHLKS  EMHNKTVSQR  FGLLLESYCR  ACGMYLKHLN  RQVEAMEKLI
721 NLTDILKQEK  KDETQKVQMK  FLVEQMRRPD  FMDALQGFLS  PLNPAHQ LGN  LRLEECRIMS
781 SAKRPLWLNW  ENPDIMSELL  FQNEIIFKN  GDDLQDMLT  LQIIRIMENI  WQNQGLDLRM
841 LPYGCLSIGD  CVGLIEVVRN  SHTIMQIQCK  GGLKGALQFN  SHTLHQWLKD  KNKGEIYDAA
901 IDLFRTRSCAG  YCVATFILGI  GDRHNSNIMV  KDDGQLFHID  FGHFLDHKKK  KFGYKRERVP
961 FVLTQDFLIV  ISKGAQECTK  TREFERFQEM  CYKAYLAIRQ  HANLFINLFS  MMLGSGMPEL
1021 QSFDDIAYIR  KTLALDKTEQ  EALEYFMKQM  NDAHGGWTT  KMDWIFHTIK  QHALN
    
```

#### Recombinant p110 $\alpha$ (E542K) nucleotide sequence:

```

1  atgcatcacc  atcaccatca  catgcctcca  agaccatcat  caggtgaact  gtggggcacc
61  cacttgatgc  cccaagaat  ctagtagaa  tgtttactac  caaatggaat  gatagtgact
121  ttagaatgcc  tccgtgaggc  tacattaata  accataaagc  atgaactatt  taaagaagca
181  agaaaatacc  cctccatca  acttcttcaa  gatgaatctt  cttacatttt  cgtaagtgtt
241  actcaagaag  cagaaagga  agaatttttt  gatgaaacaa  gacgactttg  tgaccttcgg
301  ctttttcaac  cttttttaa  agtaattgaa  ccagtaggca  accgtgaaga  aaagatcctc
361  aatcgagaaa  ttggttttgc  tatcgcatg  ccagtgtgtg  aatttgatat  ggttaaagat
421  ccagaagtac  aggacttccg  aagaaatatt  ctgaacgttt  gtaaagaagc  tgtggatcct
481  agggacctca  attcacctca  tagtagagca  atgtatgtct  atcctccaaa  tgtagaatct
541  tcaccagaat  tgccaaagca  catatataat  aaattagata  aagggcaaat  aatagtgggtg
601  atctgggtaa  tagtttctcc  aaataatgac  aagcagaagt  atactctgaa  aatcaacct
661  gactgtgtac  cagaacaagt  aattgctgaa  gcaatcagga  aaaaaactcg  aagtatgttg
721  ctatcctctg  aacaactaaa  actctgtgtt  ttagaatatc  agggcaagta  tattttaaaa
781  gtgtgtggat  gtgatgaata  cttcctagaa  aaatcctc  tgagtcagta  taagtatata
841  agaagctgta  taatgcttgg  gaggatgcc  aatttgatgt  tgatggctaa  agaaagcctt
901  tattctcaac  tgccaatgga  ctgttttaca  atgccatctt  attccagacg  catttcaca
961  gctacaccat  atatgaatgg  agaaacatct  acaaaatccc  tttgggttat  aaatagtgca
1021  ctcagaataa  aaattctttg  tgcaacctac  gtgaatgtaa  atattcgaga  cattgataag
1081  atctatgttc  gaacaggtat  ctaccatgga  ggagaaccct  tatgtgacaa  tgtgaacact
1141  caaagagtac  cttgttccaa  tcccaggtgg  aatgaatggc  tgaattatga  tatatacatt
1201  cctgatcttc  ctctgtctgc  tcgactttgc  ctttccattt  gctctgttaa  aggccgaaag
1261  ggtgctaaag  aggaacactg  tccattggca  tggggaaata  taaacttggt  tgattacaca
1321  gacactctag  tatctggaaa  aatggctttg  aatctttggc  cagtacctca  tggattagaa
    
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```
1381 gatttgc tga accctattgg tgttactgga tcaaatccaa ataaagaaac tccatgctta
1441 gaggttggagt ttgactgggt cagcagtggt gtaaagttcc cagatatgtc agtgattgaa
1501 gagcatgccca attggtctgt atcccagaaa gcaggattta gctattccca cgcaggactg
1561 agtaacagac tagctagaga caatgaatta agggaaaatg acaaagaaca gctcaaagca
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1861 tttgctgttc ggtgcttggg aaaatattta acagatgaca aactttctca gtatttaatt
1921 cagctagtac aggtcctaaa atatgaacaa ttttggata acttgcttgt gagattttta
1981 ctgaagaaag cattgactaa tcaaaggatt gggcactttt tcttttggca tttaaaatct
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3121 gaggtcttgg agtatttcat gaaacaaatg aatgatgcac atcatggtgg ctggacaaca
3181 aaaatggatt ggatcttcca cacaattaa cagcatgcat tgaactga
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## p85α Sequence Information

<b>Protein</b>	Human p85α
<b>Tags</b>	Untagged
<b>Native sequence</b>	M1 of the recombinant protein is equivalent to M1 of human p85α
<b>Accession number</b>	GenBank XM_043865

### Recombinant p85α amino acid sequence:

```

1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPDLAE
121 QFAPPDIAPP LLIKLVIAIE KKGLECESTLY RTQSSSNLAE LRQLLDLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNVIPA AVYSEMISLA PEVQSSEEIYI QLLKKLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFSS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKT RDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDEDLPHHD EKTWNVGSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNSL NVTLAYPVYA
721 QRRR

```

### Recombinant p85α nucleotide sequence:

```

1 atgagtgctg aggggtacca gtacagagcg ctgtatgatt ataaaaagga aagagaagaa
61 gatattgact tgcacttggg tgacatattg actgtgaata aagggtcctt agtagctctt
121 ggattcagtg atggacagga agccaggcct gaagaaattg gctggttaaa tggctataat
181 gaaaccacag gggaaagggg ggactttccg ggaacttacg tagaatatat tggaaagaaa
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301 tcttcgaaaa ctgaagcaga tgttgaacaa caagctttga ctctcccgga tcttgcagag
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421 aagaaaggtc tggaatgttc aactctatac agaacacaga gctccagcaa cctggcagaa
481 ttacgacagc ttcttgattg tgatacacc tccgtggact tggaaatgat cgatgtgcac
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721 cagtatattg taaaacattt cttcaagctc tctcaaacct ccagcaaaaa tctgttgaat
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901 gcacagcctg caccagcact gcctcctaaa ccacaaaac ctactactgt agccaacaac
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1201 gtggttgaat taataaacca ctaccggaat gaatctctag ctcagtataa tcccaaattg
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1321 aatattgaag ctgtagggaa aaaattacat gaatataaca ctcagtttca agaaaaaagt
1381 cgagaatatg atagattata tgaagaatat acccgccat cccaggaaat ccaaatgaaa
1441 aggacagcta ttgaagcatt taatgaaac ataaaaatat ttgaagaaca gtgccagacc
1501 caagagcggg acagcaaaga atacatagaa aagtttaaac gtgaaggcaa tgagaaagaa
1561 atacaagga ttatgataa ttatgataag ttgaagtctc gaatcagtga aattattgac

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```
1621 agtagaagaa gattggaaga agacttgaag aagcaggcag ctgagtatcg agaaattgac
1681 aaacgtatga acagcattaa accagacctt atccagctga gaaagacgag agaccaatac
1741 ttgatgtggt tgactcaaaa aggtgttcgg caaaagaagt tgaacgagtg gttgggcaat
1801 gaaaacactg aagaccaata ttcactggtg gaagatgatg aagatttgcc ccatcatgat
1861 gagaagacat ggaatgttgg aagcagcaac cgaaacaaag ctgaaaacct gttgcgaggg
1921 aagcgagatg gcacttttct tgtccgggag agcagtaaac agggctgcta tgcctgctct
1981 gtagtggtgg acggcgaagt aaagcattgt gtcataaaca aaacagcaac tggctatggc
2041 ttgccgagc cctataactt gtacagctct ctgaaagaac tggtgctaca ttaccaacac
2101 acctcccttg tgcagcaaca cgactccctc aatgtcacac tagcctacc agtatatgca
2161 cagcagaggc gatga
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Reviewed and approved by site quality representative.

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