

Certificate of Analysis

PI 3-Kinase (p110 α (E545K)/p85 α)

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-783, 14-783-K, 14-783M

Parent Lot # 1654888

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 α containing the mutation E545K and untagged, recombinant, full-length human p85 α . Co-expressed by baculovirus in Sf21 insect cells and purified using Ni²⁺/NTA-agarose.

The E545K substitution is a somatic mutation in p110 α 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 α / p85 α combined) 85.8% by SDS-PAGE and Coomassie blue staining. p110 α (E545K) MW = 125.3kDa, p85 α MW = 83.7kDa.

Specific Activity (Parent lot# 1654888): 355U/mg, where one unit of PI 3-Kinase alpha (p110 α (E545K)/p85 α (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 μ M.

Formulation: 0.789mg/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.

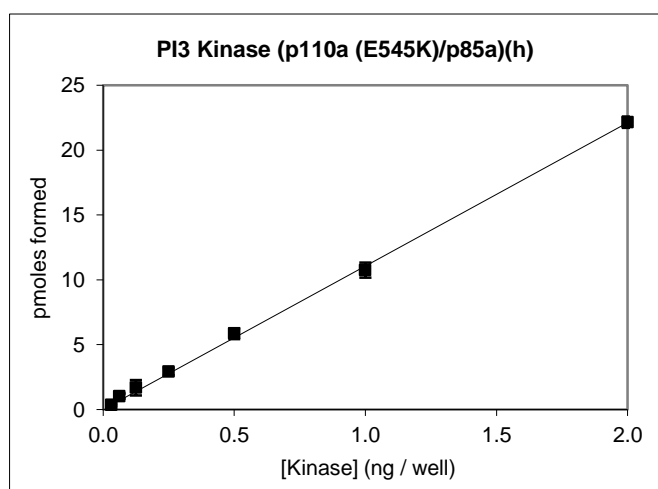
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 0.03–2ng of this enzyme phosphorylated 10 μ M phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.

MS Tryptic Fingerprint: Confirmed product identity as PI 3-Kinase (p110 α /p85 α) with the p110 α and p85 α translated sequences listed on pages three to six.



Certificate of Analysis

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).

Certificate of Analysis

p110α(E545K) Sequence Information

<u>Protein</u>	Human p110α(E545K)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human p110α
<u>Accession number</u>	GenBank U79143

Recombinant p110α(E545K) amino acid sequence:

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1  MHHHHHMP PPSSGELWGI HLMPPRILVE CLLPNGMIVT LECLREATLI TIKHELKFEA
61  RKYPLHQLLQ DESSYIFVSV TQEAEREEFF DETRRLCDLR LFPFLKLVIE PVGNREEKIL
121 NREIGFAIGM PVCEFDMVKD PEVQDFRRNI LNVCKEAVDL RDLNSPHSRA MYVYPPNVES
181 SPELPKHIYN KLDKGQIIVV IWVIVSPNND KQKYTLKINH DCVPEQVIAE AIRKKTRSML
241 LSSEQLKLCV LEYQKGYILK VCGCDEYFLE KYPLSQYKYI RSCIMLGRMP NLMLMAKESL
301 YSQLPMDCFM MPSYSRRIST ATPYMNGETS TKSLWVINSALRIKILCATY VNVNIRDIDK
361 IYVRTGIYHG GEPLCDNVNT QRVPCSNPRW NEWLNYDIYI PDLPRAARLC LSICSVKGRK
421 GAKEEHCP LAWGNINLFDYT DTLVSGKMAL NLWPVPHGLE DLLNPIGVTG SNPNETPCL
481 ELEFDWFSSV VKFPDMSVIE EHANWSVSRE AGFSYSHAGL SNRLARDNEL RENDKEQLKA
541 ISTRDPLSEI TKQEKDFLWS HRHYCVTIPE ILPKLLLSVK WNSRDEVAQM YCLVKDWPPI
601 KPEQAMELLD CNYPDMVRG FAVRCLEKYL TDDKLSQYLI QLVQVLKYEQ YLDNLLVRF
661 LKKALTNQRI GHFFFHHLKS EMHNKTVSQR FGLLLESYCR ACGMYLKHLN RQVEAMEKLI
721 NLTDLKQEK KDETQKVQMK FLVEQMRRPD FMDALQGFSL PLNPAHQ LGN LRLEECRIMS
781 SAKRPLWLNW ENPDIMSELL FQNEIIFKN GDDL RQDMLT LQIIRIMENI WQNQGLDLRM
841 LPYGCLSIGD CVGLIEVVRN SHTIMQIQCK GGLKGALQFN SHTLHQWLKD KNKGEIYDAA
901 IDLFTRSCAG YCVATFILGI GDRHNSNIMV KDDGQLFHID FGHFLDHKKK KFGYKRERP
961 FVLTQDFLIV ISKGAQECTK TREFERFQEM CYKAYLAIRQ HANLFINLFS MMLGSGMPEL
1021 QSFDDIAYIR KTLALDKTEQ EALEYFMKQM NDAHGGWTT KMDWIFHTIK QHALN

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Recombinant p110α(E545K) nucleotide sequence:

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1  atgcatcacc atccacatca catgcctcca agaccatcat caggtgaact gtggggcacc
61  cacttgatgc cccaagaat cctagtagaa tgtttactac caaatggaat gatagtgact
121 ttagaatgcc tccgtgaggc tacattaata accataaagc atgaactatt taaagaagca
181 agaaaatacc cctccatca acttcttcaa gatgaatcct cttacatttt cgtaatgttt
241 actcaagaag cagaaagga agaatttttt gatgaaacaa gacgactttg tgaccttcgg
301 ctttttcaac cttttttaa agtaattgaa ccagtaggca accgtgaaga aaagatcctc
361 aatcgagaaa ttggttttgc tatcggcatg ccagtgtgtg aatttgatat ggttaaagat
421 ccagaagtac aggacttccg aagaaatatt ctgaacgttt gtaaagaagc tgtggatcct
481 aggacacctca attcacctca tagtagagca atgtatgtct atcctccaaa tgtagaatct
541 tcaccagaat tgccaaagca catatataat aaattagata aagggcaaat aatagtggtg
601 atctgggtaa tagtttctcc aaataatgac aagcagaagt atactctgaa aatcaacct
661 gactgtgtac cagaacaagt aattgctgaa gcaatcagga aaaaaactcg aagtatgttg
721 ctatcctctg aacaactaaa actctgtgtt ttagaataatc agggcaagta tattttaaaa
781 gtgtgtggat gtgatgaata cttcctagaa aaatattctc tgagtcagta taagtatata
841 agaagctgta taatgcttgg gaggatgcc aatttgatgt tgatggctaa agaaagcctt
901 tattctcaac tgccaatgga ctgttttaca atgccatcct attccagacg catttcaca
961 gctacaccat atatgaatgg agaaacatct acaaaatccc tttgggttat aatagtgca
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1081 atctatgttc gaacaggat ctaccatgga ggagaaccct tatgtgacaa tgtgaacact
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1201 cctgatcttc ctcgtctgc tcgacttgc ctttccattt gctctgttaa aggccgaaag
1261 ggtgctaaag aggaactg tccattggca tggggaaata taaacttggt tgattacaca

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1321 gacactctag tatctggaaa aatggctttg aatctttggc cagtaccta tggattagaa
1381 gatttgctga accctattgg tgttactgga tcaaatacaa ataaagaaac tccatgctta
1441 gagttggagt ttgactgggt cagcagtgtg gtaaagtcc cagatatgtc agtgattgaa
1501 gagcatgcc aattggtctgt atcccagaaa gcaggattta gctattccca cgcaggactg
1561 agtaacagac tagctagaga caatgaatta agggaaaatg acaaagaaca gctcaaagca
1621 atttctacac gagatcctct ctctgaaatc actaagcagg agaaagattt tctatggagt
1681 cacagacact attgtgtaac tatccccgaa attctacca aattgcttct gtctgttaaa
1741 tggaaattcta gagatgaagt agcccagatg tattgcttgg taaaagattg gcctccaatc
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1921 cagctagtac aggtcctaaa atatgaacaa tatttggata acttgcttgt gagattttta
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2641 agccacacac tacatcagtg gctcaaagac aagaacaaag gagaaatata tgatgcagcc
2701 attgacctgt ttacacgttc atgtgctgga tactgtgtag ctaccttcat tttgggaatt
2761 ggagatcgtc acaatagtaa catcatgggtg aaagacgatg gacaactgtt tcatatagat
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3001 catgccaatc tcttcataaa tcttttctca atgatgcttg gctctggaat gccagaacta
3061 caatcttttg atgacattgc atacattcga aagaccctag ctttagataa aactgagcaa
3121 gaggcttttg agtatttcat gaaacaaatg aatgatgcac atcatggtgg ctggacaaca
3181 aaaatggatt ggatcttcca cacaataaa cagcatgcat tgaactga

Certificate of Analysis

p85α Sequence Information

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

Recombinant p85α amino acid sequence:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDAE
121 QFAPPDIAPP LLIKLVEAIE KKGLECSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEIYI QLLKKLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDDLTFSS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK S REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDELPHHD EKTWNVGSSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNYLSS LKELVLHYQH TSLVQHNSL NVTLAYPVYA
721 QRRR
    
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Recombinant p85α nucleotide sequence:

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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
241 aaaatctcgc ctccacacc aaagccccgg ccacctcggc ctcttcctgt tgcaccaggt
301 tcttcgaaaa ctgaagcaga tgttgaacaa caagcttga ctctcccgga tcttgcagag
361 cagtttgccc ctctgacat tgccccgctt cttcttatca agctcgtgga agccattgaa
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1621 agtagaagaa gattggaaga agacttgaag aagcaggcag ctgagtatcg agaaattgac
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1741 ttgatgtggt tgactcaaaa aggtgttcgg caaaagaagt tgaacgagtg gttgggcaat
1801 gaaaacactg aagaccaata ttcactgggtg gaagatgatg aagatttgcc ccatcatgat
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1921 aagcgagatg gcacttttct tgtccgggag agcagtaaac agggctgcta tgcctgctct
1981 gtagtgggtg acggcgaagt aaagcattgt gtcataaaca aaacagcaac tggctatggc
2041 tttgccgagc cctataactt gtacagctct ctgaaagaac tggtgctaca ttaccaacac
2101 acctcccttg tgcagcacia cgactccctc aatgtcacac tagcctacc agtatatgca
2161 cagcagaggc gatga
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Reviewed and approved by site quality representative.

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