

Certificate of Analysis

PI 3-Kinase (p110 α (H1047R)/p85 α)

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-792, 14-792-K, 14-792M

Parent Lot # WAE0047

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 human p110 α full length, containing the mutation H1047R, and untagged, recombinant, human p85 α full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

The H1047R substitution is a somatic mutation in p110 α that has been associated with tumours of the colon, stomach, breast 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. (Samuels Y. *et al.*, Science, (2004); **304**: 554; Kang S. *et al.*, PNAS, (2005); **102**: 802-807 and Zhao J.J. *et al.*, PNAS, (2005); **102**:18443-18448).

Purity (p110 α and p85 α combined) 89% by SDS-PAGE and Coomassie blue staining. p110 α MW = 129kDa, p85 α MW = 83.6kDa.

Specific Activity (Parent lot# WAE0047): 214U/mg, where one unit of PI 3-Kinase (p110 α (H1047R)/p85 α) 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: 0.39mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

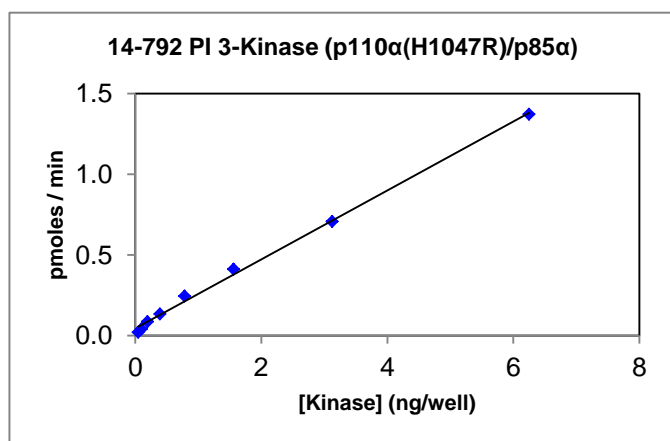
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

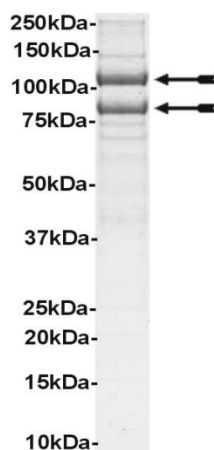
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.05 – 6.25ng 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 and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of active PI 3-Kinase (p110 α (H1047R)/p85 α).

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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).

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p110α(H1047R) Sequence Information

<u>Protein</u>	Human p110α(H1047R)
<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(H1047R) amino acid sequence:

```

1  MHHHHHMP  RPSSGELWGI  HLMPPRILVE  CLLPNGMIVT  LECLREATLI  TIKHELKFEA
61  RKYPLHQLLQ  DESSYIFVSV  TQEAEREFF  DETRRLCDLR  LFPFLKLVIE  PVGNREEKIL
121  NREIGFAIGM  PVCFDMVKD  PEVQDFRRI  LNVCKEAVDL  RDLNSPHSRA  MYVYPPNVES
181  SPELPKHIYN  KLDKGQIIV  IWVIVSPND  KQKYLKINH  DCVPEQVIAE  AIRKTRSM
241  LSSEQLKLCV  LEYQKYLK  VCGCDEYFL  KYPLSQKYI  RSCIMLGRMP  NLMLMAKESL
301  YSQLPMDCF  MPSYSRRIS  TATPYMNGE  TKSLWVINS  A  LRIKILCATY  VNVNIRDIDK
361  IYVRTGIYHG  GEPLCDNV  T  QRVPCSNPRW  NEWLNYDIY  I  PDLPRAARLC  LSICSVKGRK
421  GAKEEHCPLA  WGNINLFDY  T  DTLVSGKMAL  NLWPVPHGLE  DLLNPIGVTG  SNPKNKTPCL
481  ELEFDWFSSV  VKFPDMSV  IE  EHANWSVSRE  AGFSYSHAGL  SNRLARDNEL  RENDKEQLKA
541  ISTRDPLSEI  TEQEKDFL  WS  HRHYCVTIPE  ILPKLLLSVK  WNSRDEVAQM  YCLVKDWPPI
601  KPEQAMELLD  CNYPDMVR  G  FAVRCLEKYL  TDDKLSQYLI  QLVQVLKYEQ  YLDNLLVRF
661  LKKALTNQRI  GHFFFWHL  KS  EMHNKTVSQR  FGLLLESYCR  ACGMYLKHLN  RQVEAMEKLI
721  NLTDLKQEK  KDEQKQVM  K  FLVEQMRRPD  FMDALQGF  LS  PLNPAHQ  LGN  LRLEECRIMS
781  SAKRPLWLNW  ENPDIMSEL  L  FQNEIIFKN  GDDLQDMLT  LQIIRIMENI  WQNQGLDLRM
841  LPYGCLSIGD  CVGLIEVVR  N  SHTIMQIQCK  GGLKGALQFN  SHTLHQWLKD  KNKGEIYDAA
901  IDLFTRSCAG  YCVATFIL  GI  GDRHNSNIMV  KDDGQLFHID  FGHFLDHKKK  KFGYKRERVP
961  FVLTQDFLIV  ISKGAQECT  K  TREFERFQEM  CYKAYLAIRQ  HANLFINLFS  MMLGSGMPEL
1021  QSFDDIAYIR  KTLALDKTE  Q  EALEYFMKQM  NDARHGGWTT  KMDWIFHTIK  QHALN

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Recombinant p110α(H1047R) nucleotide sequence:

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1  atgcatcacc  atcaccatca  catgcctcca  agaccatcat  caggtgaact  gtggggcatc
61  cacttgatgc  cccaagaat  cctagtagaa  tgtttactac  caaatggaat  gatagtgact
121  ttagaatgcc  tccgtgaggc  tacattaata  accataaagc  atgaactatt  taaagaagca
181  agaaaatacc  cctccatca  acttcttcaa  gatgaatctt  cttacatttt  cgtaatgttt
241  actcaagaag  cagaaagga  agaatttttt  gatgaaaca  gacgactttg  tgaccttcgg
301  ctttttcaac  cttttttaa  agtaattgaa  ccagtaggca  accgtgaaga  aaagatcctc
361  aatcgagaaa  ttggttttgc  tatcggcatg  ccagtgtgtg  aatttgatat  ggttaaagat
421  ccagaagtac  aggacttccg  aagaatatt  ctgaactgtt  gtaaagaagc  tgtggatctt
481  agggacctca  attcacctca  tagtagagca  atgtatgtct  atcctccaaa  tgtagaatct
541  tcaccagaat  tgccaaagca  catatataat  aaattagata  aagggcaaat  aatagtgtgtg
601  atctgggtaa  tagtttctc  aaataatgac  aagcagaagt  atactctgaa  aatcaacct
661  gactgtgtac  cagaacaagt  aattgctgaa  gcaatcagga  aaaaaactcg  aagtatgttg
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781  gtgtgtggat  gtgatgaata  cttcctagaa  aaatctctc  tgagtcagta  taagtatata
841  agaagctgta  taatgcttgg  gaggatgcc  aatttgatgt  tgatggctaa  agaaagcctt
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961  gctacaccat  atatgaatgg  agaaacatct  acaaaatccc  tttgggttat  aaatagtgca
1021  ctcaagaata  aattctttg  tgcaacctac  gtgaatgtaa  atattcgaga  cattgataag
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1141  caaagagtac  cttgttcaa  tcccagggtg  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 gatttgctga accctattgg tgttactgga tcaaatccaa ataaagaac tccatgctta
1441 gagttggagt ttgactgggt cagcagtgtg gtaaagtcc cagatatgtc agtgattgaa
1501 gagcatgcc aattggtctgt atcccagaa gcaggattta gctattcca cgcaggactg
1561 agtaacagac tagctagaga caatgaatta agggaaaatg acaaagaaca gctcaaagca
1621 atttctacac gagatcctct ctctgaaatc actgagcagg agaaagattt tctatggagt
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1801 aaacctgaac aggctatgga acttctggac tgtaattacc cagatcctat ggttcgaggt
1861 tttgctgttc ggtgcttggg aaaatattta acagatgaca aactttctca gtatttaatt
1921 cagctagtac aggtcctaaa atatgaaca ttttggata acttgcttgt gagatttta
1981 ctgaagaag agtactaa tcaaaggat gggcactttt tcttttggca tttaaaatct
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2701 attgacctgt ttacacgttc atgtgctgga tactgtgtag ctaccttcat tttgggaatt
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2881 tttgttttga cacaggattt cttaatagtg attagtaaag gagcccaaga atgcacaaag
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3061 caatcttttg atgacattgc atacattcga aagaccctag ctttagataa aactgagcaa
3121 gaggcttttg agtatttcat gaaacaaatg aatgatgcac gtcattggtg ctggacaaca
3181 aaaatggatt ggatcttcca cacaattaa cagcatgcat tgaactga

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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 GFSGDQEARP EEIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDAE
121 QFAPPDIAPP LLIKLVEAIE KKGLECASTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEIYI QLLKKLIRSP SIPHQYWLTTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNMMNSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDDLTFSS VVELINHYRN ESQAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK SREYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKRTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDELPHHD EKTWNVGSSN RNKAENLLRG KRDTGFLVRE 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 tgacatatg actgtgaata aagggtcctt agtagctctt
121 ggattcagtg atggacagga agccaggcct gaagaaattg gctggttaaa tggctataat
181 gaaaccacag gggaaagggg ggactttccg ggaacttacg tagaatatat tggaaaggaaa
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301 tcttcgaaaa ctgaagcaga tgttgaacaa caagcttga ctctcccgga tcttgcagag
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481 ttacgacagc ttcttgattg tgatacacc tccgtggact tggaaatgat cgatgtgcac
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781 gcaagagtac tctctgaaat tttcagcctt atgcttttca gattctcagc agccagctct
841 gataaactg aaaacctcat aaaagtata gaaattttaa tctcaactga atggaatgaa
901 cgacagcctg caccagcact gcctcctaaa ccacaaaac ctactactgt agccaacaac
961 ggtatgaata acaatatgtc cttacaagat gctgaatggt actggggaga tatctcgagg
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1561 atacaagga ttatgcataa ttatgataag ttgaagtctc gaatcagtga aattattgac
1621 agtagaagaa gattggaaga agacttgaag aagcaggcag ctgagtatcg agaaattgac
    
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1681 aaacgtatga acagcattaa accagacctt atccagctga gaaagacgag agaccaatac
1741 ttgatgtggt tgactcaaaa aggtgttcgg caaaagaagt tgaacgagtg gttgggcaat
1801 gaaaacactg aagaccaata ttcactgggtg gaagatgatg aagatttgcc ccatcatgat
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2041 ttgcccagc cctataactt gtacagctct ctgaaagaac tggctgctaca ttaccaacac
2101 acctcccttg tgcagcacia cgactccctc aatgtcacac tagcctaccc agtatatgca
2161 cagcagaggc gatga
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Reviewed and approved by site quality representative.

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