eurofins | Discovery Services

Certificate of Analysis

PI 3-Kinase (p110α(H1047R)/p85α)

(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 p110α full length, containing the mutation H1047R, and untagged, recombinant, human p85α full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
The H1047R substitution is a somatic mutation in p110α 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 (p110α and p85α combined) 89% by SDS-PAGE and Coomassie blue staining. p110α MW = 129kDa, p85α MW = 83.6kDa.

Specific Activity (Parent lot# WAE0047): 214U/mg, where one unit of PI 3-Kinase (p110α(H1047R)/p85α) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate (PIP3) formed per minute at room temperature with a final ATP concentration of 100µM.

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.

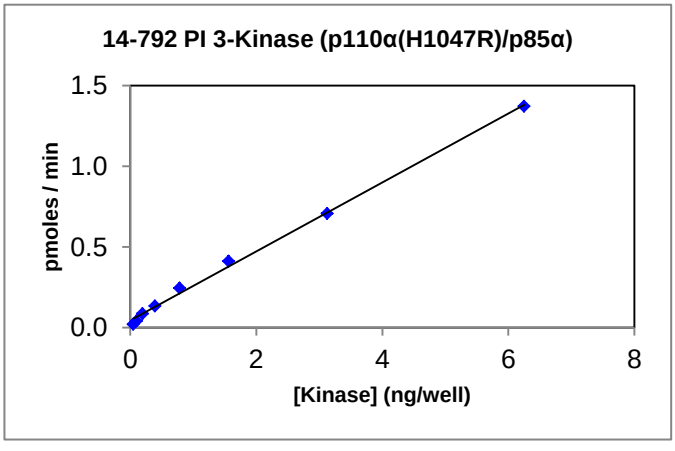
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

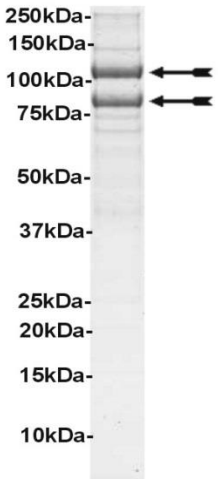
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.05 – 6.25ng of this enzyme phosphorylated 10µM phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.



MS Tryptic Fingerprint: Confirmed product identity as PI 3-Kinase (p110α/p85α) with the p110α and p85α translated sequences listed on pages three and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active PI 3-Kinase (p110α(H1047R)/p85α) .

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

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).

eurofins | Discovery Services

Certificate of Analysis

p110α(H1047R) Sequence Information

Protein Human p110α(H1047R)

Tags *N*-terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1 of human p110α

Accession number GenBank U79143

Recombinant p110α(H1047R) amino acid sequence:

```
   1 MHHHHHHMPP RPSSGELWGI HLMPPRILVE CLLPNGMIVT LECLREATLI TIKHELFKEA
  61 RKYPLHQLLQ DESSYIFVSV TQEAEREEFF DETRRLCDLR LFQPFLKVIE PVGNREEKIL
 121 NREIGFAIGM PVCEFDMVKD PEVQDFRRNI LNVCKEAVDL RDLNSPHSRA MYVYPPNVES
 181 SPELPKHIYN KLDKGQIIVV IWVIVSPNND KQKYTLKINH DCVPEQVIAE AIRKKTRSML
 241 LSSEQLKLCV LEYQGKYILK VCGCDEYFLE KYPLSQYKYI RSCIMLGRMP NLMLMAKESL
 301 YSQLPMDCFT MPSYSRRIST ATPYMNGETS TKSLWVINSA LRIKILCATY VNVNIRDIDK
 361 IYVRTGIYHG GEPLCDNVNT QRVPCSNPRW NEWLNYDIYI PDLPRAARLC LSICSVKGRK
 421 GAKEEHCPLA WGNINLFDYT DTLVSGKMAL NLWPVPHGLE DLLNPIGVTG SNPNKETPCL
 481 ELEFDWFSSV VKFPDMSVIE EHANWSVSRE AGFSYSHAGL SNRLARDNEL RENDKEQLKA
 541 ISTRDPLSEI TEQEKDFLWS HRHYCVTIPE ILPKLLLSVK WNSRDEVAQM YCLVKDWPPI
 601 KPEQAMELLD CNYPDPMVRG FAVRCLEKYL TDDKLSQYLI QLVQVLKYEQ YLDNLLVRFL
 661 LKKALTNQRI GHFFFWHLKS EMHNKTVSQR FGLLLESYCR ACGMYLKHLN RQVEAMEKLI
 721 NLTDILKQEK KDETQKVQMK FLVEQMRRPD FMDALQGFLS PLNPAHQLGN LRLEECRIMS
 781 SAKRPLWLNW ENPDIMSELL FQNNEIIFKN GDDLRQDMLT LQIIRIMENI WQNQGLDLRM
 841 LPYGCLSIGD CVGLIEVVRN SHTIMQIQCK GGLKGALQFN SHTLHQWLKD KNKGEIYDAA
 901 IDLFTRSCAG YCVATFILGI GDRHNSNIMV KDDGQLFHID FGHFLDHKKK KFGYKRERVP
 961 FVLTQDFLIV ISKGAQECTK TREFERFQEM CYKAYLAIRQ HANLFINLFS MMLGSGMPEL
1021 QSFDDIAYIR KTLALDKTEQ EALEYFMKQM NDARHGGWTT KMDWIFHTIK QHALN
```

Recombinant p110α(H1047R) nucleotide sequence:

```
   1 atgcatcacc atcaccatca catgcctcca agaccatcat caggtgaact gtggggcatc
  61 cacttgatgc ccccaagaat cctagtagaa tgtttactac caaatggaat gatagtgact
 121 ttagaatgcc tccgtgaggc tacattaata accataaagc atgaactatt taaagaagca
 181 agaaaatacc ccctccatca acttcttcaa gatgaatctt cttacatttt cgtaagtgtt
 241 actcaagaag cagaaaggga agaatttttt gatgaaacaa gacgactttg tgaccttcgg
 301 ctttttcaac ccttttttaaa agtaattgaa ccagtaggca accgtgaaga aaagatcctc
 361 aatcgagaaa ttggttttgc tatcggcatg ccagtgtgtg aatttgatat ggttaaagat
 421 ccagaagtac aggacttccg aagaaatatt ctgaacgttt gtaaagaagc tgtggatctt
 481 agggacctca attcacctca tagtagagca atgtatgtct atcctccaaa tgtagaatct
 541 tcaccagaat tgccaaagca catatataat aaattagata aagggcaaat aatagtggtg
 601 atctgggtaa tagtttctcc aaataatgac aagcagaagt atactctgaa aatcaaccat
 661 gactgtgtac cagaacaagt aattgctgaa gcaatcagga aaaaaactcg aagtatgttg
 721 ctatcctctg aacaactaaa actctgtgtt ttagaatatc agggcaagta tattttaaaa
 781 gtgtgtggat gtgatgaata cttcctagaa aaatatcctc tgagtcagta taagtatata
 841 agaagctgta taatgcttgg gaggatgccc aatttgatgt tgatggctaa agaaagcctt
 901 tattctcaac tgccaatgga ctgttttaca atgccatctt attccagacg catttccaca
 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 ctcgtgctgc tcgactttgc ctttccattt gctctgttaa aggccgaaag
1261 ggtgctaaag aggaacactg tccattggca tggggaaata taaacttgtt tgattacaca
1321 gacactctag tatctggaaa aatggctttg aatctttggc cagtacctca tggattagaa
```

Certificate of Analysis

```
1381 gatttgctga accctattgg tgttactgga tcaaatccaa ataaagaaac tccatgctta
1441 gagttggagt ttgactggtt cagcagtgtg gtaaagttcc cagatatgtc agtgattgaa
1501 gagcatgcca attggtctgt atcccgagaa gcaggattta gctattccca cgcaggactg
1561 agtaacagac tagctagaga caatgaatta agggaaaatg acaaagaaca gctcaaagca
1621 atttctacac gagatcctct ctctgaaatc actgagcagg agaaagattt tctatggagt
1681 cacagacact attgtgtaac tatccccgaa attctaccca aattgcttct gtctgttaaa
1741 tggaattcta gagatgaagt agcccagatg tattgcttgg taaaagattg gcctccaatc
1801 aaacctgaac aggctatgga acttctggac tgtaattacc cagatcctat ggttcgaggt
1861 tttgctgttc ggtgcttgga aaaatattta acagatgaca aactttctca gtatttaatt
1921 cagctagtac aggtcctaaa atatgaacaa tatttggata acttgcttgt gagattttta
1981 ctgaagaaag cattgactaa tcaaaggatt gggcactttt tcttttggca tttaaaatct
2041 gagatgcaca ataaaacagt tagccagagg tttggcctgc ttttggagtc ctattgtcgt
2101 gcatgtggga tgtatttgaa gcacctgaat aggcaagtcg aggcaatgga aaagctcatt
2161 aacttaactg acattctcaa acaggagaag aaggatgaaa cacaaaaggt acagatgaag
2221 tttttagttg agcaaatgag gcgaccagat ttcatggatg ctctacaggg ctttctgtct
2281 cctctaaacc ctgctcatca actaggaaac ctcaggcttg aagagtgtcg aattatgtcc
2341 tctgcaaaaa ggccactgtg gttgaattgg gagaacccag acatcatgtc agagttactg
2401 tttcagaaca atgagatcat ctttaaaaat ggggatgatt tacggcaaga tatgctaaca
2461 cttcaaatta ttcgtattat ggaaaatatc tggcaaaatc aaggtcttga tcttcgaatg
2521 ttaccttatg gttgtctgtc aatcggtgac tgtgtgggac ttattgaggt ggtgcgaaat
2581 tctcacacta ttatgcaaat tcagtgcaaa ggcggcttga aaggtgcact gcagttcaac
2641 agccacacac tacatcagtg gctcaaagac aagaacaaag gagaaatata tgatgcagcc
2701 attgacctgt ttacacgttc atgtgctgga tactgtgtag ctaccttcat tttgggaatt
2761 ggagatcgtc acaatagtaa catcatggtg aaagacgatg gacaactgtt tcatatagat
2821 tttggacact ttttggatca caagaagaaa aaatttggtt ataaacgaga acgtgtgcca
2881 tttgttttga cacaggattt cttaatagtg attagtaaag gagcccaaga atgcacaaag
2941 acaagagaat ttgagaggtt tcaggagatg tgttacaagg cttatctagc tattcgacag
3001 catgccaatc tcttcataaa tcttttctca atgatgcttg gctctggaat gccagaacta
3061 caatcttttg atgacattgc atacattcga aagaccctag ccttagataa aactgagcaa
3121 gaggctttgg agtatttcat gaaacaaatg aatgatgcac gtcatggtgg ctggacaaca
3181 aaaatggatt ggatcttcca cacaattaaa cagcatgcat tgaactga
```

eurofins | Discovery Services

Certificate of Analysis

p85α Sequence Information

Protein Human p85α

Tags Untagged

Native sequence M1 of the recombinant protein is equivalent to M1 of human p85α

Accession number GenBank XM_043865

Recombinant p85α amino acid sequence:

```
   1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNGYN
  61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPDLAE
 121 QFAPPDIAPP LLIKLVEAIE KKGLECSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
 181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEYI QLLKKLIRSP SIPHQYWLTL
 241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
 301 RQPAPALPPK PPKPTTVANN GMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
 361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFSS VVELINHYRN ESLAQYNPKL
 421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEKS REYDRLYEEY TRTSQEIQMK
 481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
 541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
 601 ENTEDQYSLV EDDEDLPHHD EKTWNVGSSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
 661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNDSL NVTLAYPVYA
 721 QQRR
```

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 tggaaggaaa
 241 aaaatctcgc ctcccacacc aaagccccgg ccacctcggc ctcttcctgt tgcaccaggt
 301 tcttcgaaaa ctgaagcaga tgttgaacaa caagctttga ctctcccgga tcttgcagag
 361 cagtttgccc ctcctgacat tgccccgcct cttcttatca agctcgtgga agccattgaa
 421 aagaaaggtc tggaatgttc aactctatac agaacacaga gctccagcaa cctggcagaa
 481 ttacgacagc ttcttgattg tgatacaccc tccgtggact tggaaatgat cgatgtgcac
 541 gttttggctg acgctttcaa acgctatctc ctggacttac caaatcctgt cattccagca
 601 gccgtttaca gtgaaatgat ttctttagct ccagaagtac aaagctccga agaatatatt
 661 cagctattga agaagcttat taggtcgcct agcatacctc atcagtattg gcttacgctt
 721 cagtatttgt taaaacattt cttcaagctc tctcaaacct ccagcaaaaa tctgttgaat
 781 gcaagagtac tctctgaaat tttcagccct atgcttttca gattctcagc agccagctct
 841 gataatactg aaaacctcat aaaagttata gaaattttaa tctcaactga atggaatgaa
 901 cgacagcctg caccagcact gcctcctaaa ccaccaaaac ctactactgt agccaacaac
 961 ggtatgaata acaatatgtc cttacaagat gctgaatggt actggggaga tatctcgagg
1021 gaagaagtga atgaaaaact tcgagataca gcagacggga ccttttggt acgagatgcg
1081 tctactaaaa tgcatggtga ttatactctt acactaagga aaggggaaa taacaaatta
1141 atcaaaatat ttcatcgaga tgggaaatat ggcttctctg acccattaac cttcagttct
1201 gtggttgaat taataaacca ctaccggaat gaatctctag ctcagtataa tcccaaattg
1261 gatgtgaaat tactttatcc agtatccaaa taccaacagg atcaagttgt caaagaagat
1321 aatattgaag ctgtagggaa aaaattacat gaatataaca ctcagtttca agaaaaaagt
1381 cgagaatatg atagattata tgaagaatat acccgcacat cccaggaaat ccaaatgaaa
1441 aggacagcta ttgaagcatt taatgaaacc ataaaaatat ttgaagaaca gtgccagacc
1501 caagagcggt acagcaaaga atacatagaa aagtttaaac gtgaaggcaa tgagaaagaa
1561 atacaaagga ttatgcataa ttatgataag ttgaagtctc gaatcagtga aattattgac
1621 agtagaagaa gattggaaga agacttgaag aagcaggcag ctgagtatcg agaaattgac
```

eurofins | Discovery Services

Certificate of Analysis

```
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 tttgccgagc cctataactt gtacagctct ctgaaagaac tggtgctaca ttaccaacac
2101 acctcccttg tgcagcacaa cgactccctc aatgtcacac tagcctaccc agtatatgca
2161 cagcagaggc gatga
```

Reviewed and approved by site quality representative.