

Certificate of Analysis

PI 3-Kinase (p110 α (H1047R)/p85 α), murine (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-787, 14-787-K, 14-787M

Parent Lot # D7SN036U

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 murine p110 α full length, containing the mutation H1047R, and untagged, recombinant, murine p85 α full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

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) 89.9% by SDS-PAGE and Coomassie blue staining. p110 α MW = 129kDa, p85 α MW = 83.6kDa.

Specific Activity (Parent lot# D7SN036U): 438U/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: 1.013mg/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.

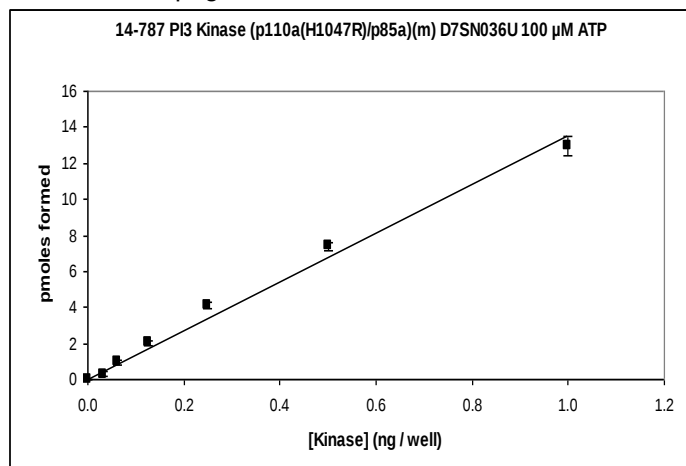
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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: 0.03–1ng of this enzyme phosphorylated 10 μ M phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.



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



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

p110 α (H1047R) Sequence Information

Protein	Murine p110 α (H1047R)
Tags	N-terminal 6His
Native sequence	M37 of the recombinant protein is equivalent to M1 of mouse p110 α
Accession number	GenBank BC089038

Recombinant p110 α (H1047R) amino acid sequence:

```

1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELFREAR KYPLHQLLQD ETSYIFVSVT QEAEREFFD
121 ETRRLCDLRL FQPFVKVIEP VGNREEKILN REIGFVIGMP VCEFDVMKDP EVQDFRRNIL
181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQIIVVI WVIVSPNNDK
241 QKYTLKINH D CVPEQVIAEA IRKKTRSMML SSEQLKLCVL EYQGYILKV CGCDEYFLEK
301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
361 KSLWVINSAL RIKILCATYV NVNIRDIDKI YVRTGIYHGG EPLCDNVNTQ RVPCSNPRWN
421 EWLNYDIYIP DLPRAARLCL SICSVKGRKG AKEEHCPLAW GNINLFDYTD TLVSGKMALN
481 LWPVPHGLED LLNPIGVTGS NPNKETPCLE LEFDWFSSVV KFPDMSVIEE HANWSVSREA
541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSEIT EQEKDFLWSH RHYCVTIPEI
601 LPKLLLSVKW NSRDEVAQMY CLVKDWPPIK PEQAMELLDC NYPDPMVRSF AVRACLEKYLT
661 DDKLSQYLIQ LVQVLKYEYQ LDNLLVRFLK KALTNRIG HFFFVHLKSE MHNKTVSQRF
721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEKK DETQKVQMKF LVEQMRQPDF
781 MDALQGFLSP LNPALQLGNL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIIRIMENIW QNQGLDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKGALQFNS HTLHQWLKDK NKGEIYDAAI DLFTRSCAGY CVATFILGIG DRHNSNIMVK
961 DDGQLFHIDF GHFLDHKKKK FGYKRERVPF VLTQDFLIVI SKGAQEYTKT REFERFQEMC
1021 YKAYLAIRQH ANLFINLFSM MLGSGMPQLQ SFDDIAYIRK TLALDKTEQE ALEYFTKQMN
1081 DARHGGWTTK MDWIFHTIKQ HALN

```

Recombinant p110 α (H1047R) nucleotide sequence:

```

1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gcctccacga
121 ccatcttcgg gtgaactgtg gggcatccac ttgatgcccc cacgaatcct agtggaatgt
181 ttactcccca atggaatgat agtgacttta gaatgcctcc gtgaggccac actcgtcacc
241 atcaaacatg aactgttcag agaggccagg aaataccctc tccatcagct tctgcaagac
301 gaaacttctt acattttcgt aagtgtcacc caagaagcag aaagggaaga attttttgat
361 gaaacaagac gactttgtga cttcggctt tttcaaccct ttttaaaagt tattgaacca
421 gtaggcaacc gtgaagaaaa gatcctcaat cgagaaattg gttttgttat tggcatgcca
481 gtgtgtgaat ttgatatggt taaagatcca gaagtccaag actttcgaag gaacattctg
541 aatgtttgca aagaagctgt ggacctgcgg gatctcaact cgcctcatag cagagcaatg
601 tatgtctacc ctccaaatgt cgagcttccc ccagaactgc caaagcacat ctacaacaag
661 ttagataaag gacaaatcat agtggtgatt tgggtaatag tctctccaaa caacgacaag
721 cagaagtaca ctctgaagat caatcatgac tgtgtgccag agcaagtcac tgctgaagcc
781 atcaggaaaa agactcggag catgttggtg tcctctgagc agctgaaact ctgtgtctta
841 gaatatcagg gcaagtatat tctgaaagtg tgtggctgtg acgaatactt cctggaaaag
901 taccctctga gtcagtacaa gtacataaga agctgtataa tgctggggag gatgcccaac
961 ttgatgctga tggccaaaga aagcctatac tctcagctgc cgattgatag cttcaccatg
1021 ccgtcatact ccaggcgcac ctccacagcc acaccctaca tgaatggaga gacatctacg
1081 aaatccctct gggtcataaa tagtgcgctc agaataaaaa ttctttgtgc aacctatgta
1141 aatgtaaata ttcgagacat tgataagatc tatgttcgaa caggatatcta ccatggagga

```

Certificate of Analysis

```

1201 gaacccttat gtgacaatgt gaacactcaa agagtacctt gttccaatcc taggtggaat
1261 gaatggctga attatgatat atacattcct gatcttcctc gtgctgcgcg cctttgcctt
1321 tcaatctgct ctgttaaagg ccgaaagggt gctaaggagg agcactgtcc gttggcctgg
1381 ggaacataaa acttgtttga ttatacagac accctagtgt ccgggaaaat ggctttgaat
1441 ctctggcctg taccgcatgg gttagaagat ctgctgaacc ctattggtgt tactgggtca
1501 aatccaaata aagaaactcc atgcttagag ttggagtttg attggttcag cagtgtggtg
1561 aagtttccag acatgtctgt gatcgaagaa catgccaat gggtccgtgtc ccgagaagct
1621 ggattcagtt actcccatag aggactgagt aacagactag ccagagacaa tgagttaaga
1681 gaaaatgaca aggaacagct ccgagcactt tgcacccggg acccactatc tgaaatcact
1741 gaacaagaga aagacttcct atggagccac agacactact gcgtaactat tcctgaaatc
1801 ctacccaaat tgcttctgtc tgtcaagtgg aattccagag acgaagtggc ccagatgtac
1861 tgcttagtaa aagattggcc tccaatcaaa ccagagcaag ccatggaact cctggactgt
1921 aactatccag atcctatggt tcggagtttt gctgttcggt gcttagaaaa atatttaaca
1981 gatgacaaac tttctcagta cctcattcaa cttgtacagg tcttaaaata tgaacagtat
2041 ttggataacc tgcttgtgag atttttactc aagaaagcat tgacaaatca aaggattggc
2101 cattttttct tttggcattt aaaatctgag atgcacaata agactgtcag tcagaggttt
2161 ggctgtctat tggagtccta ctgccgtgcc tgtgggatgt atctgaagca cctgaacaga
2221 caagtagagg ccatggagaa gctcatcaac ctaacggaca tccttaagca ggagaagaag
2281 gatgagacac aaaagggtaca gatgaagttt ttggttgaac agatgagaca gccagacttc
2341 atggatgctt tgcagggttt tctgtccctt ctgaatcctg ctcaccaact aggaaacctc
2401 aggcttgaag agtgtcgaat tatgtcctct gcaaaaaggc cactgtggtt gaattgggag
2461 aaccagaca tcatgtcaga gctactgttt cagaacaatg agatcatctt taaaaatggc
2521 gacgacttac ggcaagatat gttaaccctt cagatcatcc gaatcatgga gaacatctgg
2581 caaaaccaag gccttgacct tcgcatgcta ctttatggct gtctatccat tggggactgt
2641 gtgggtctca tcgagggtgt gagaaactct cacaccatca tgcaaatcca gtgcaaagga
2701 ggctgaagg gggcgctgca gttcaacagc cacacactgc atcaatggct caaggacaag
2761 aacaagggcg agatatatga tgcagccatt gacctgttca ctcggtcctg cgctgggtac
2821 tgcgtggcaa cttttatctt gggaattgga gaccggcaca acagcaacat catggtgaaa
2881 gatgacggac agctgttcca tatagatttt gggcactttt tggatcacia gaagaaaaaa
2941 tttggctata agcgggaacg tgtgccattt gtgttgacac aggatttctt gattgtgatt
3001 agtaaggagg cacaagagta caccaagacc agagagtttg agaggtttca ggagatgtgt
3061 tacaaggctt acctagcaat tcggcagcat gccaatctct tcatcaacct tttttcaatg
3121 atgcttggct ctggaatgcc agaactacaa tcttttgatg acattgcata tatccgaaag
3181 actctagcct tggacaaaac tgagcaagaa gctttggaat atttcacaaa gcaaatgaat
3241 gatgcacgtc atggtggatg gacgacaaaa atggattgga tcttccacac catcaagcag
3301 catgctttga actaa

```

Certificate of Analysis

p85α Sequence Information

<u>Protein</u>	Murine p85α
<u>Tags</u>	Untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of murine p85α
<u>Accession number</u>	GenBank NM_001077495

Recombinant p85α amino acid sequence:

```

1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EDIGWLNGYN
61 ETTGERGDFP GTYVEYIGRK RISPTPKPR PPRPLPVAPG SSKTEADTEQ QALPLPDIAE
121 QFAPPDVAPP LLIKLLEAIE KKGLECSLY RTQSSSNPAE LRQLDCDAA SVDLEMIDVH
181 VLADAFKRYL ADLPNPVIPV AVYNEMMSLA QELQSPEDCI QLLKKLIRLP NIPHQCWLT
241 QYLLKHFFKL SQASSKNLLN ARVLSEIFSP VLFRFPAASS DNTEHLIKAI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN SMNNNMSLQD AEWYWGDISR EEVNEKL RDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDDLTFNS VVELINHYRN ESQAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDELPHHD EKTWNVGSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
661 VVDGEVKHC VINKTATGYG FAEPYNYLYSS LKELVLHYQH TSLVQHNSL NVTLAYPVYA
721 QQR

```

Recombinant p85α nucleotide sequence:

```

1 atgagtgtctg aggggtacca gtacagagca ctgtacgact acaagaagga gcgagaggaa
61 gacattgacc tacacctggg ggacatactg actgtgaata aaggctcctt agtggcactt
121 ggattcagtg atggccagga agcccgccct gaagatatgt gctggttaaa tggctacaat
181 gaaaccactg gggagagggg agactttcca ggaacttacg ttgaatacat tggaaaggaaa
241 agaatttcac ccctactccc caagcctcgg cccctcgcac cgcttcctgt tgctccgggt
301 tcttcaaaaa ctgaagctga cacggagcag caagcgttgc ccttcctga cctggccgag
361 cagtttgccc ctctgatgtg tgccccgcct ctcttataaa agctcctgga agccattgag
421 aagaaaggac tggaaatgtc gactctatac agaacacaaa gctccagcaa ccctgcagaa
481 ttacgacagc ttcttgattg tgatgccgcg tcagtggact tggagatgat cgacgtacac
541 gtcttagcag atgctttcaa acgctatctc gccgacttac caaatcctgt cattcctgta
601 gctgtttaca atgagatgat gtctttagcc caagaactac agagccctga gactcgtatc
661 cagctgttga agaagctcat tgattgcctt aatatacttc atcagtgttg gcttacgctt
721 cagtatttgc tcaagcattt tttcaagctc tctcaagcct ccagcaaaaa ccttttgaat
781 gcaagagtcc tctctgagat tttcagcccc gtgcttttca gatttccagc cgccagctct
841 gataatactg aacacctcat aaaagcgata gagattttta tctcaacgga atggaatgag
901 agacagccag caccagcact gcccccaaaa ccaccaagc ccactactgt agccaacaac
961 agcatgaaca acaatatgtc cttgcaggat gctgaatggg actggggaga catctcaagg
1021 gaagaagtga atgaaaaact ccgagacact gctgatggga cctttttggg acgagacgca
1081 tctactaaaa tgcacggcga ttactactct acactaagga aaggaggaaa taacaaatta
1141 atcaaaatct ttcaccgtga tggaaaatat ggcttctctg atccattaac cttcaactct
1201 gtggttgagt taataaacca ctaccggaat gactctttag ctcagtacaa cccaagctg
1261 gatgtgaagt tgctctaccc agtgtccaaa taccagcagg atcaagttgt caaagaagat
1321 aatattgaag ctgtagggaa aaaattacat gaatataata ctcaatttca agaaaaaagt
1381 cggaatatg atagattata tgaggagtac acccgtaact cccaggaaat ccaaatgaaa
1441 agaacggcta tcgaagcatt taatgaaacc ataaaaatat ttgaagaaca atgccaacc
1501 caggagcggg acagcaaaga atacatagag aagtttaaac gcgaaggcaa cgagaaagaa
1561 attcaaagga ttatgcataa ccatgataag ctgaagtcgc gtatcagtga gatcattgac

```

Certificate of Analysis

```

1621 agtaggagga ggttgaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac
1681 aaacgcatga acagtattaa gccggacctc atccagttga gaaagacaag agaccaatac
1741 ttgatgtggc tgacgcagaa aggtgtgcgg cagaagaagc tgaacgagtg gctggggaat
1801 gaaaataccg aagatcaata ctccctggta gaagatgatg aggatttgcc ccaccatgac
1861 gagaagacgt ggaatgtcgg aagcagcaac cgaaacaaag cggagaacct attgcgaggg
1921 aagcgagacg gcactttcct tgtccgggag agcagtaagc agggctgcta tgcctgctcc
1981 gtagtggtag acggcgaagt caagcattgc gtcattaaca agactgccac cggctatggc
2041 ttgcccagc cctacaacct gtacagctcc ctgaaggagc tggtgctaca ttatcaacac
2101 acctccctcg tgcagcacia tgactccctc aatgtcacac tagcataccc agtatatgca
2161 caacagaggc gataa

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services