

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.

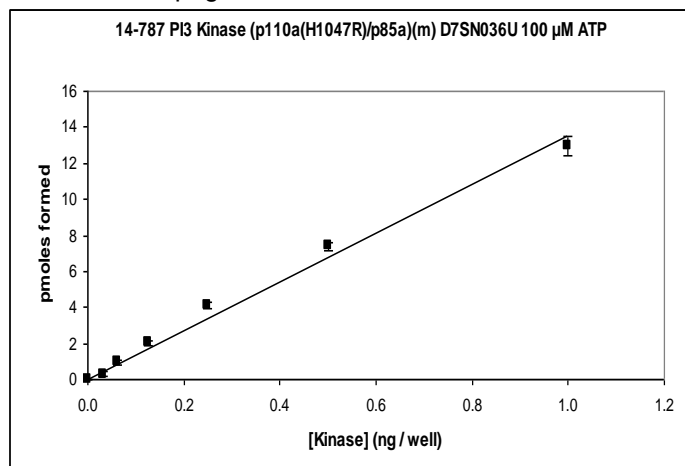
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.



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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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p110 α (H1047R) Sequence Information

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

Recombinant p110 α (H1047R) amino acid sequence:

```

1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELFRERAR KYPLHQLLQD ETSYIFVSVT QEAEREFFFD
121 ETRRLCDLRL FQPFLKVIIEP 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 AKEEHCLAW GNINLFDYTD TLVSGKMALN
481 LWPVPHGLED LLNPIGVTGS NPNKETPCLE LEFDWFSSVV KFPDMSVIEE HANWSVSREA
541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSEIT EQEKDFLWSH RHYCVTIPEI
601 LPKLLLSVKW NSRDEVAQMY CLVKDWPPIK PEQAMELLDC NYPDPMVRSF AVRCKEYLYT
661 DDKLSQYLIQ LVQVLKYEQY LDNLLVRFLL KKALTNQRIG HFFFWHLKSE MHNKTVSQRF
721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEKK DETQKVQMKF LVEQMRQPDF
781 MDALQGFLSP LNPALQGLNL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIIRIMENIW QNQGLDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKQALQFNS HTLHQWLKDK NKGEIYDAAI DLFTRSCAGY CVATFILGIG DRHNSNIMVK
961 DDGQLFHIDF GHFLDHKKKK FGYKRERVPF VLTQDFLIVI SKGAQEYTKT REFERFQEMC
1021 YKAYLAIRQH ANLFINLFSM MLGSGMPELQ SFDDIAYIRK TLALDKTEQE ALEYFTKQMN
1081 DARHGGWTTK MDWIFHTIKQ HALN
    
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Recombinant p110 α (H1047R) nucleotide sequence:

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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 aaataacctc tccatcagct tctgcaagac
301 gaaacttctt acattttcgt aagtgtcacc caagaagcag aaagggaaga attttttgat
361 gaaacaagac gactttgtga ctttcggctt tttcaaccct ttttaaaagt tattgaacca
421 gtaggcaacc gtgaagaaaa gatcctcaat cgagaaattg gttttgttat tggcatgcca
481 gtgtgtgaat ttgatatggt taaagatcca gaagtccaag actttcgaag gaacattctg
541 aatgtttgca aagaagctgt ggacctgagg gatctcaact cgcctcatag cagagcaatg
601 tatgtctacc ctccaatgt cgagtctcc ccagaactgc caagcacat 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 caggatctca ccatggagga
    
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1201 gaacccttat gtgacaatgt gaacactcaa agagtacctt gttccaatcc taggtggaat
1261 gaatggctga attatgatat atacattcct gatcttcctc gtgctgcgcg cctttgcctt
1321 tcaatctgct ctgttaaagg ccgaaagggt gctaaggagg agcactgtcc gttggcctgg
1381 ggaacataa acttgtttga ttatacagac accctagtgt ccgggaaaat ggctttgaat
1441 ctctggcctg taccgcatgg gttagaagat ctgctgaacc ctattgggtg tactgggtca
1501 aatccaaata aagaaactcc atgcttagag ttggagtgtg attggttcag cagtgtgggtg
1561 aagtttccag acatgtctgt gatcgaagaa catgccaaat ggtccgtgtc ccgagaagct
1621 ggattcagtt actcccatac aggactgagt aacagactag ccagagacia tgagttaaga
1681 gaaaatgaca aggaacagct ccgagcactt tgcacccggg acccactatc tgaatcact
1741 gaacaagaga aagacttcct atggagccac agacactact gcgtaactat tcctgaaatc
1801 ctacccaaat tgcttctgtc tgtcaagtgg aattccagag acgaagtggc ccagatgtac
1861 tgcttagtaa agattggcc tccaatcaaa ccagagcaag ccattggaact cctggactgt
1921 aactatccag atcctatggt tcggagtgtt gctgttcggt gcttagaaaa atatttaaca
1981 gatgacaaac tttctcagta cctcattcaa cttgtacagg tcttaaaata tgaacagtat
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2221 caagtagagg ccatggagaa gctcatcaac ctaacggaca tccttaagca ggagaagaag
2281 gatgagacac aaaaggatca gatgaagttt ttggttgaac agatgagaca gccagacttc
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2701 ggcttgaagg gggcgtgca gttcaacagc cacacactgc atcaatggct caaggacaag
2761 aacaagggcg agatataatga tgcagccatt gacctgttca ctcggctcctg cgctgggtac
2821 tgcgtggcaa cttttatctt gggatttga gaccggcaca acagcaacat catggtgaaa
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3121 atgcttggct ctggaatgcc agaactacia tcttttgatg acattgcata tatccgaaag
3181 actctagcct tggacaaaac tgagcaagaa gctttggaat atttcacaaa gcaaatgaat
3241 gatgcacgtc atggtggatg gacgacaaaa atggattgga tcttccacac catcaagcag
3301 catgctttga actaa

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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:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EDIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK RISPTPKPR PPRPLPVAPG SSKTEADTEQ QALPLPDLAE
121 QFAPPDVAPP LLIKLLEAIE KKGLECSTLY RTQSSSNPAE LRQLLDCDAA SVDLEMIDVH
181 VLADAFKRYL ADLPNPVIPV AVYNEMMSLA QELQSPEDCI QLLKKLIRLP NIPHQCWLTL
241 QYLLKHFFKL SQASSKNLLN ARVLSEIFSP VLFRFPAASS DNTEHLIKAI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN SMNNSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNKL IKIFHRDGKY GFSDP LTFNS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKLLH EYNTQFQEK S REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKQV R QKKLNEWLGN
601 ENTEDQYSLV EDDELPHHD EKTWNVGSN RNKAENLRG 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 gtacagagca ctgtacgact acaagaagga gcgagaggaa
61 gacattgacc tacacctggg ggacatactg actgtgaata aaggctcctt agtggcactt
121 ggattcagtg atggccagga agccccgctt gaagatattg gctggttaaa tggctacaat
181 gaaaccactg gggagagggg agactttcca ggaacttacg ttgaatacat tggaaaggaaa
241 agaatttcac cccctactcc caagcctcgg cccccctgac cgcttcctgt tgctccgggt
301 tcttcaaaaa ctgaagctga cacggagcag caagcgttgc ccttcctga cctggccgag
361 cagtttgccc ctctgatgt tgccccgctt ctcttataa agctcctgga agccattgag
421 aagaaaggac tggaaatggt gactctatac agaacacaaa gctccagcaa cctgcagaa
481 ttacgacagc ttcttgattg tgatgcccgg tcagtggact tggagatgat cgagctacac
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721 cagtatttgc tcaagcattt tttcaagctc tctcaagcct ccagcaaaaa ccttttgaat
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901 agacagccag caccagcact gcccccaaaa ccaccaagc ccactactgt agccaacaac
961 agcatgaaca acaatatgtc cttgcaggat gctgaatggg actgggggaga catctcaagg
1021 gaagaagtga atgaaaaact ccgagacact gctgatggga cctttttggg acgagacgca
1081 tctactaaaa tgcacggcga ttactactctt aactaagga aaggaggaaa taacaaatta
1141 atcaaaatct ttcaccgtga tggaaaatat ggcttctctg atccattaac cttcaactct
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1321 aatattgaag ctgtagggaa aaaattacat gaatataata ctcaatttca agaaaaaagt
1381 cggaatatg atagattata tgaggagtac acccgactt cccaggaaat ccaaatgaaa
1441 agaacggcta tcgaagcatt taatgaaacc ataaaaatat ttgaagaaca atgccaacc
1501 caggagcggg acagcaaaga atacatagag aagttaaacc gcgaaggcaa cgagaaagaa
1561 attcaagga ttatgcataa ccatgataag ctgaagtcgc gtatcagtga gatcattgac
    
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1621 agtaggagga ggttgaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac
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1741 ttgatgtggc tgacgcagaa aggtgtgcgg cagaagaagc tgaacgagtg gctggggaat
1801 gaaaataccg aagatcaata ctccctggta gaagatgatg aggatttgcc ccaccatgac
1861 gagaagacgt ggaatgtcgg aagcagcaac cgaaacaaag cggagaacct attgagaggg
1921 aagcgagacg gcactttcct tgtccgggag agcagtaagc agggctgcta tgcctgctcc
1981 gtagtggtag acggcgaagt caagcattgc gtcattaaca agactgccac cggctatggc
2041 tttgccgagc cctacaacct gtacagctcc ctgaaggagc tggtgctaca ttatcaacac
2101 acctccctcg tgcagcaaa tgactccctc aatgtcacac tagcatatccc agtatatgca
2161 caacagaggc gataa
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