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Certificate of Analysis

PI 3-Kinase (p110δ/p85α) mouse

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-789, 14-789-K, 14-789M
Parent Lot # 1604633

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 mouse p110delta full length and untagged, recombinant, mouse p85alpha full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose.
Purity (p110δ and p85α combined) 76% by SDS-PAGE and Coomassie blue staining. p110δ MW = 124kDa, p85α MW = 84kDa.

Specific Activity (Parent lot# 1604633): 343U/mg, where one unit of PI 3-Kinase (p110δ/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: 0.774mg/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.

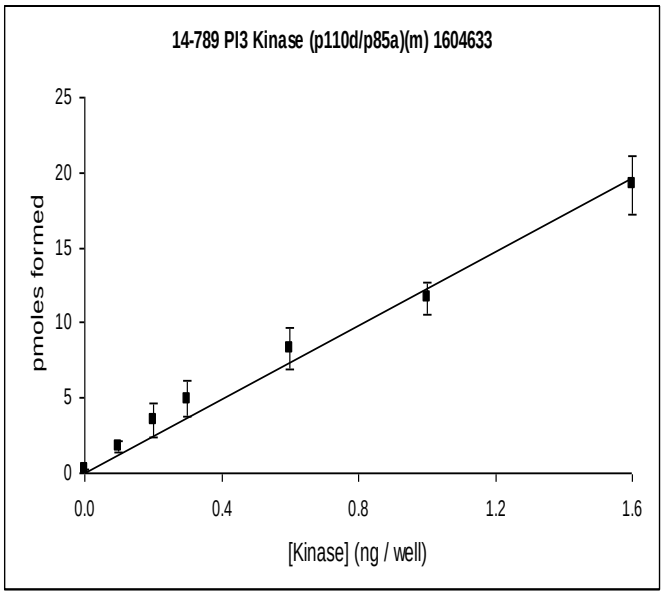
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

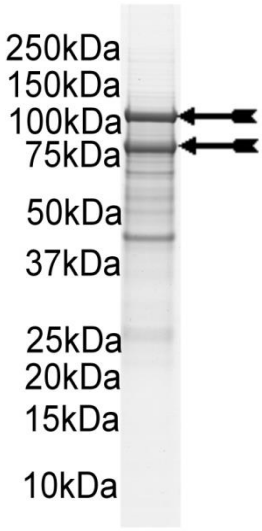
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.0–1.6ng 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 (p110δ/p85α) with the p110δ 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 (p110δ/p85α)

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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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p110δ Sequence Information

Protein Mouse p110δ

Tags *N*-terminal 6His

Native sequence M37 of the recombinant sequence is equivalent to M1 of mouse p110δ

Accession number GenBank NM_008840

Recombinant p110δ amino acid sequence:

```
   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPG VDCPMEFWTK EESQSVVVDF
  61 LLPTGVYLNF PVSRNANLST IKQVLWHRAQ YEPLFHMLSD PEAYVFTCVN QTAEQQELED
 121 EQRRLCDIQP FLPVLRLVAR EGDRVKKLIN SQISLLIGKG LHEFDSLRDP EVNDFRTKMR
 181 QFCEEAAAHR QQLGWVEWLQ YSFPLQLEPS ARGWRAGLLR VSNRALLVNV KFEGSEESFT
 241 FQVSTKDMPL ALMACALRKK ATVFRQPLVE QPEEYALQVN GRHEYLYGNY PLCHFQYICS
 301 CLHSGLTPHL TMVHSSSILA MRDEQSNPAP QVQKPRAKPP PIPAKKPSSV SLWSLEQPFS
 361 IELIEGRKVN ADERMKLVVQ AGLFHGNEML CKTVSSSEVN VCSEPVWKQR LEFDISVCDL
 421 PRMARLCFAL YAVVEKAKKA RSTKKKSKKA DCPIAWANLM LFDYKDQLKT GERCLYMWPS
 481 VPDEKGELLN PAGTVRGNPN TESAAALVIY LPEVAPHPVY FPALEKILEL GRHGERGRIT
 541 EEEQLQLREI LERRGSGELY EHEKDLVWKM RHEVQEHFPE ALARLLLVTK WNKHEDVAQM
 601 LYLLCSWPEL PVLSALELLD FSFPDCYVGS FAIKSLRKLT DDELFQYLLQ LVQVLKYESY
 661 LDCELTKFLL GRALANRKIG HFLFWHLRSE MHVPSVALRF GLIMEAYCRG STHHMKVLMK
 721 QGEALSKLKA LNDFVKVSSQ KTTKPQTKEM MHMCMRQETY MEALSHLQSP LDPSTLLEEV
 781 CVEQCTFMDS KMKPLWIMYS SEEAGSAGNV GIIFKNGDDL RQDMLTLQMI QLMDVLWKQE
 841 GLDLRMTPYG CLPTGDRTGL IEVVLHSDTI ANIQLNKSNM AATAAFNKDA LLNWLKSKNP
 901 GEALDRAIEE FTLSCAGYCV ATYVLGIGDR HSDNIMIRES GQLFHIDFGH FLGNFKTKFG
 961 INRERVPFIL TYDFVHVIQQ GKTNNSEKFE RFRGYCERAY TILRRHGLLF LHLFALMRAA
1021 GLPELSCSKD IQYLKDSLAL GKTEEEALKH FRVKFNEALR ESWKTKVNWL AHNVSKDNRQ
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Recombinant p110δ nucleotide sequence:

```
   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gccccctggg
 121 gtggactgcc ccatggagtt ctggaccaaa gaggagagcc agagcgtggt tgttgacttc
 181 ttgctgccca caggggtcta cttgaacttc cccgtgtccc gcaatgccaa cctcagcacc
 241 atcaagcagg tgctgtggca ccgtgcacag tatgagccac tcttccacat gctcagtgac
 301 cccgaggcct atgtgttcac ctgtgtgaac cagacggcgg agcagcagga gttggaggat
 361 gagcagcgga ggctgtgcga catccagccc ttcctgcccg tgctgcgcct cgtggcccga
 421 gaggggacc gcgtgaagaa gctcattaac tcccagatca gcctcctcat tggcaaaggt
 481 ctccatgagt ttgattccct gcgggacccg gaagtaaacg acttccgcac taagatgcgc
 541 cagttttgtg aagaggctgc tgctcaccgc cagcagctgg gctgggtgga atggctgcag
 601 tacagcttcc ccctgcagct ggagccctca gcaaggggtt ggcgggccgg cttattgcgt
 661 gtcagcaacc gagccctgct ggtcaacgtg aagttcgagg gcagtgagga gagcttcacc
 721 ttccaggtat ccaccaagga catgcccctg gcactgatgg cctgtgccct ccgaaaaaag
 781 gccacagtgt tccggcagcc tctggtggag cagcctgagg aatatgccct gcaggtgaac
 841 gggaggcacg aatacctcta cggcaactac ccgctctgcc actttcagta catctgcagc
 901 tgcctacaca gcgggctgac ccctcatctg accatggtcc actcctcctc catccttgct
 961 atgcgggatg agcagagcaa tcctgccccc caagtacaga aaccacgtgc caaacctccc
1021 ccgatccctg ccaagaagcc ctcctctgtg tccctgtggt ccctggaaca gccattctcc
1081 attgagctga tcgagggccg aaaagtgaat gctgacgagc ggatgaagct ggttgttcag
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1201 gtatgctcag agcccgtgtg gaagcagcga ctggagttcg atatcagcgt ctgtgacctc
1261 ccgcgcatgg ctcgactctg ttttgctctc tatgccgtcg tggagaaggc taagaaggca
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1321 cgctccacaa agaagaagtc taagaaggcg gactgcccca tcgcttgggc caacctcatg
1381 ctattcgact acaaagatca gctcaagacg ggggagcgct gcctctacat gtggccctct
1441 gtcccagatg agaagggaga gctgctgaat cctgcgggta cagtgcgcgg gaaccccaac
1501 acggagagtg ccgctgccct ggtcatctac ctgcctgagg tggcccccca ccctgtgtac
1561 ttccccgctc tggagaagat cctggagctg gggcgtcacg gggagcgtgg gcgcatcacg
1621 gaggaggagc agctgcagct gcgggagatc ctggaacggc ggggatccgg ggaactgtac
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1861 tttagctttc ccgactgcta cgtgggctcc ttcgccatca agtcccttcg gaagctgacg
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2701 ggggaggccc tggatcgggc cattgaggaa ttcaccctct cctgtgctgg ctactgtgtg
2761 gccacatatg ttctgggcat cggtgaccgg cacagcgaca acatcatgat cagagagagt
2821 gggcagctct tccacattga ttttggccac tttctgggga acttcaagac caagtttgga
2881 atcaaccgag agcgcgtccc cttcattctc acctacgact ttgtccacgt gatccagcag
2941 gggaagacta acaacagtga gaagtttgaa aggttccgcg gctactgtga acgagcctat
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3121 gggaagacgg aggaagaggc gctaaagcac ttccgggtga agttcaacga agctctccga
3181 gaaagctgga aaaccaaagt caactggctg gcgcacaatg tgtccaagga taaccgacag
3241 taa
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p85α Sequence Information

Protein Mouse p85α

Tags Untagged

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

Accession number GenBank NM_001077495

Recombinant p85α amino acid sequence:

```
  1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EDIGWLNGYN
 61 ETTGERGDFP GTYVEYIGRK RISPPTPKPR 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 SMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFNS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEKS REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDEDLPHHD EKTWNVGSSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNDSL NVTLAYPVYA
721 QQRR
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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
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 181 gaaaccactg gggagagggg agactttcca ggaacttacg ttgaatacat tggaaggaaa
 241 agaatttcac cccctactcc caagcctcgg ccccctcgac cgcttcctgt tgctccgggt
 301 tcttcaaaaa ctgaagctga cacggagcag caagcgttgc cccttcctga cctggccgag
 361 cagtttgccc ctcctgatgt tgccccgcct ctccttataa agctcctgga agccattgag
 421 aagaaaggac tggaatgttc gactctatac agaacacaaa gctccagcaa ccctgcagaa
 481 ttacgacagc ttcttgattg tgatgccgcg tcagtggact tggagatgat cgacgtacac
 541 gtcttagcag atgctttcaa acgctatctc gccgacttac caaatcctgt cattcctgta
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 961 agcatgaaca acaatatgtc cttgcaggat gctgaatggt actggggaga catctcaagg
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1441 agaacggcta tcgaagcatt taatgaaacc ataaaaatat ttgaagaaca atgccaaacc
1501 caggagcggt acagcaaaga atacatagag aagtttaaac gcgaaggcaa cgagaaagaa
1561 attcaaagga ttatgcataa ccatgataag ctgaagtcgc gtatcagtga gatcattgac
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1621 agtaggagga ggttggaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac
1681 aaacgcatga acagtattaa gccggacctc atccagttga gaaagacaag agaccaatac
1741 ttgatgtggc tgacgcagaa aggtgtgcgg cagaagaagc tgaacgagtg gctggggaat
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2041 tttgccgagc cctacaacct gtacagctcc ctgaaggagc tggtgctaca ttatcaacac
2101 acctccctcg tgcagcacaa tgactccctc aatgtcacac tagcataccc agtatatgca
2161 caacagaggc gataa
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Reviewed and approved by site quality representative.