

Certificate of Analysis

PI3 Kinase (p110δ/p85α)

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-604, 14-604-K, 14-604M

Parent Lot # 2195161

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, full-length, human p110δ and untagged, recombinant, full length, human p85α. Coexpressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 98.0% by SDS-PAGE and Coomassie blue staining. P110δ MW = 121.6kDa, p85α MW = 83.7kDa.

Specific Activity (Parent lot# 2195161): 293U/mg, where one unit of PI 3-kinase (p110δ/p85α) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate formed per minute at 22°C with a final ATP concentration of 100μM.

Formulation: 2.734mg/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 6 months 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.

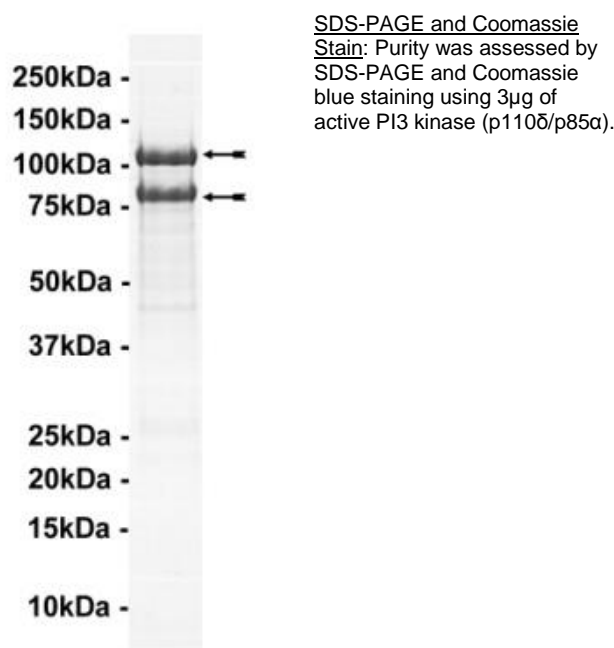
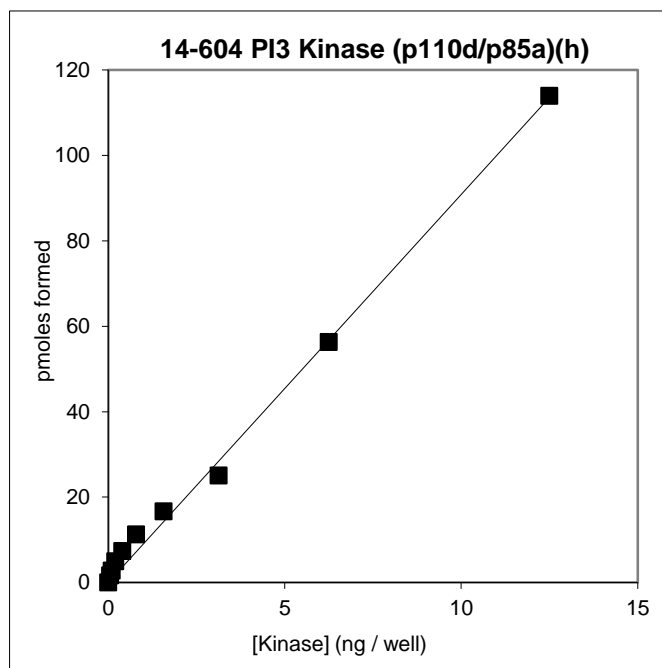
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 0–13ng of this enzyme phosphorylated 10μM phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two

MS Tryptic Fingerprint: Confirmed product identity as p110δ/p85α with the 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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P110δ Sequence Information

<u>Protein</u>	Human p110δ
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M16 of the recombinant protein is equivalent to M1 of human p110δ
<u>Accession number</u>	GenBank NM_005026. The recombinant protein contains the amino acid substitution N253S with respect to this accession number. This conflict is reported in GenBank Y10055 and U57843. The residue coordinates in the native sequence are given.

Recombinant p110δ amino acid sequence:

```

1  MHHHHHHEFK  GLRRRMPPGV  DCPMEFWTKE  ENQSVVDFL  LPTGVYLNFP  VSRNANLSTI
61  KQLLWHRAQY  EPLFHMLSGP  EAYVFTCIHQ  TAEQQELEDE  QRRLCDVQPF  LPVLRVLVARE
121 GDRVKKLINS  QISLLIGKGL  HEFDSLCDPE  VNDFRAKMCQ  FCEAAAARRQ  QLGWEAWLQY
181 SFPLQLEPSA  QTWGPGLRL  PNRALLVNK  FEGSEESFTF  QVSTKDVPLA  LMACALRKKA
241 TVFRQPLVEQ  PEDYTLQVNG  RHEYLGSYP  LCQFQYICSC  LHSGLTPHLT  MVHSSSILAM
301 RDEQSNPAPQ  VQKPRAKPPP  IPAKKPSSVS  LWSLEQPFRI  ELIQGSKVNA  DERMKLVVQA
361 GLFHGNEMLC  KTVSSSEVSV  CSEPVMKQRL  EFDINICDLP  RMARLCFALY  AVIEKAKKAR
421 STKKKSKKAD  CPIAWANLML  FDYKDQLKTG  ERCLYMWPSV  PDEKGELLNP  TGTVRSNPNT
481 DSAAALLICL  PEVAPHPVYY  PALEKILELG  RHSECVHVTE  EEQLQLREIL  ERRGSGELYE
541 HEKDLVWKLK  HEVQEHFPEA  LARLLLVTKW  NKHEDVAQML  YLLCSWPELP  VLSALELLDF
601 SFPDCHVGSF  AIKSLRKLTD  DELFQYLLQL  VQVLKYESYL  DCELTKFLLD  RALANRKIGH
661 FLFWHLRSEM  HVPVALRFG  LILEAYCRGS  THMKVLMKQ  GEALSCLKAL  NDFVKLSSQK
721 TPKPQTKELM  HLCMRQEAYL  EALSHLQSPL  DPSTLLAECV  VEQCTFMDSK  MKPLWIMYSN
781 EEAGSGGSVG  IIFKNGDDL  QDMLTLQMIQ  LMDVLWKQEG  LDLRMTPYGC  LPTGDRTGLI
841 EVVLRSDTIA  NIQLNKSMA  ATAAFNKDAL  LNLKSKNPG  EALDRAIEEF  TLSCAGYCVA
901 TYVLGIGDRH  SDNIMIRESG  QLFHIDFGHF  LGNFKTKFGI  NRERVPFILT  YDFVHVIQQG
961 KTNNSEKFER  FRGYCERAYT  ILRRHGLLFL  HLFALMRAAG  LPELSCSKDI  QYLKDSLALG
1021 KTEEEALKHF  RVKFNEALRE  SWKTKVNWLA  HNVSKDNRQ
    
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Recombinant p110δ nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattcaaa  ggcctacgtc  gacgaatgcc  ccctgggggtg
61  gactgcccc  tgaattctg  gaccaaggag  gagaatcaga  gcgttggtgt  tgacttcctg
121  ctgcccacag  gggctctact  gaacttcctt  gtgtcccgca  atgccaacct  cagcaccatc
181  aagcagctgc  tgtggcaccg  cgcccagtat  gagccgctct  tccacatgct  cagtggcccc
241  gaggcctatg  tgttcacctg  catcaaccag  acagcggagc  agcaagagct  ggaggacgag
301  caacggcgtc  tgtgtgacgt  gcagcccttc  ctgcccgtcc  tgcgcctggt  ggcccgtgag
361  ggcgaccgcg  tgaagaagct  catcaactca  cagatcagcc  tcctcatcgg  caaaggcctc
421  cacgagtttg  actccttggt  cgaccagaaa  gtgaacgact  ttcgcgccaa  gatgtgccaa
481  ttctgcgagg  aggcggccgc  ccgccggcag  cagctgggct  gggaggcctg  gctgcagtac
541  agtttcccc  tgcagctgga  gccctcggct  caaacctggg  ggcctggtac  cctgcggctc
601  ccgaaccggg  cccttctggt  caacgttaag  tttgagggca  gcgaggagag  cttcaccttc
661  caggtgtcca  ccaaggacgt  gccgctggcg  ctgatggcct  gtgccctcgc  gaagaaggcc
721  acagtgttcc  ggcagccgct  ggtggagcag  ccggaagact  acacgctgca  ggtgaacggc
781  aggcatgagt  acctgatgg  cagctaccgc  ctctgccagt  tccagtacat  ctgcagctgc
841  ctgcacagtg  ggttgacccc  tcacctgacc  atggtccatt  cctcctccat  cctcgccatg
901  cgggatgagc  agagcaacc  tgccccccag  gtccagaaac  cgcgtgccaa  accacctccc
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1261 tccaccaaga agaagtccaa gaaggcggac tgccccattg cctgggcaa cctcatgctg
1321 tttgactaca aggaccagct taagaccggg gaacgctgcc tctacatgtg gccctccgtc
1381 ccagatgaga agggcgagct gctgaacccc acgggcactg tgcgcagtaa ccccaacacg
1441 gatagcgccg ctgccctgct catctgcctg cccgaggtgg ccccgacacc cgtgtactac
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2941 atcctgcggc gccacgggct tctcttctc cacctcttg ccctgatgcg ggcggcaggc
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3121 agctggaaaa ccaaagtgaa ctggctggcc cacaacgtgt ccaaagcaa caggcagtag
    
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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:

```

1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSGDQEARP EEIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDAE
121 QFAPPDIAPP LLIKLVEAIE KKGLECESTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEIYI QLLKKLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNKL IKIFHRDGKY GFSDDLTFSS VVELINHYRN ESQAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKLLH EYNTQFQEK SREYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKRTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDELPHHD EKTWNVGSSN RNKAENLRG KRDGTFLVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNYLSS LKELVLHYQH TSLVQHNSL NVTLAYPVYA
721 QQR

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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
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1621 agtagaagaa gattggaaga agacttgaag aagcaggcag ctgagtatcg agaaattgac

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1801 gaaaacactg aagaccaata ttcactgggt gaagatgatg aagatttgcc ccatcatgat
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2041 tttgccgagc cctataactt gtacagctct ctgaaagaac tgggtgctaca 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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