

Certificate of Analysis

PI3 Kinase (p110 β /p85 α), active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-603, 14-603-K, 14-603M
Parent Lot # 1994929

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 p110 β and untagged, recombinant, full length, human p85 α . Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 72.9% by SDS-PAGE and Coomassie blue staining. P110 β MW = 124kDa, p85 α MW = 83.7kDa.

Specific Activity (Parent lot# 1994929): 144U/mg, where one unit of PI3 Kinase (p110 β), active activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate formed per minute at 22°C with a final ATP concentration of 100 μ M.

Formulation: 0.534mg/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.

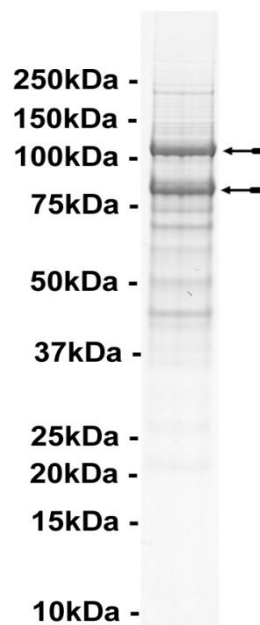
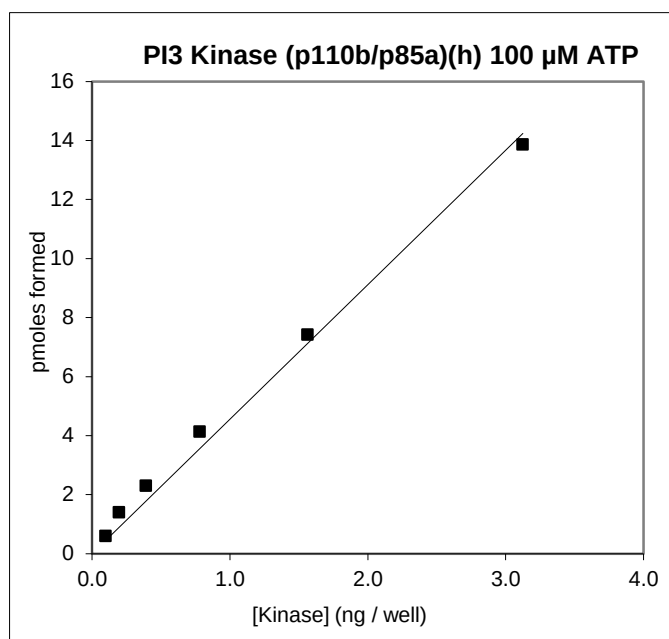
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 0.1–3.1ng of this enzyme phosphorylated 10 μ M phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.

MS Tryptic Fingerprint: Confirmed identity as PI3 Kinase (p110 β) with the translated sequence listed on pages three and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of PI3 kinase (p110 β), active.

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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 β Sequence Information

<u>Protein</u>	Human p110 β
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M10 of the recombinant protein is equivalent to M1 of human p110 β
<u>Accession number</u>	GenBank NM_006219

Recombinant p110 β amino acid sequence:

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1  MHHHHHHEFM CFSFIMPPAM ADILDIWAVD SQIASDGSIP VDFLLPTGIY IQLEVPREAT
61  ISYIKQMLWK QVHNYPMFNL LMDIDSYMFA CVNQTAVYEE LEDETRRLCD VRPFLPVLKL
121 VTRSCDPGEK LDSKIGVLIG KGLHEFDSLK DPEVNEFRRK MRKFSEEKIL SLVGLSWMDW
181 LKQTYYPEHE PSIPENLEDK LYGGKLIVAV HFENCQDVFS FQVSPNMNPI KVNELAIQKR
241 LTIHGKEDEV SPYDYVLQVS GRVEYVFGDH PLIQFYIRN CVMNRALPHF ILVECKIKK
301 MYEQEMIAIE AAINRNSSNL PLPLPPKKTR IISHVWENNN PFQIVLVKGN KLNTEETVKV
361 HVRAGLFHGT ELLCKTIVSS EVSGKNDHIW NEPLEFDINI CDLPRMARLC FAVYAVLDKV
421 KTKKSTKTIN PSKYQTIRKA GKVHYPVAWV NTMVDFDKGQ LRTGDIILHS WSSFPDELEE
481 MLNPMGTVQT NPYTENATAL HVKFPENKKQ PYYYPPFDKI IEKAAEIAS DSANVSSRGG
541 KKFLPVLKEI LDRDPLSQLC ENEMDLIWT L RQDCREIFPQ SLPKLLLSIK WNKLEDVAQL
601 QALLQIWPKL PPRALELLD FNYPDQYVRE YAVGCLRQMS DEELSQYLLQ LVQVLKYEPF
661 LDCALSRFL L ERALGNRRIG QFLFWHLRSE VHIPAVSVQF GVILEAYCRG SVGHMKVLSK
721 QVEALNKLKT LNSLIKLNAV KLNRAKGKEA MHTCLKQSAY REALSDLQSP LNPCVILSEL
781 YVECKYKMS KMKPLWL VYN NKVFGEDSVG VIFKNGDDL R QDMLTLQMLR LMDLLWKEAG
841 LDLRMLPYGC LATGDRSGLI EVVSTSETIA DIQLNSSNVA AAAAFNKDAL LNWKEYNSG
901 DDLDR AIEEF T LSCAGYCV SYVLGIGDRH SDNIMVKKTG QLFHIDFGHI LGNFKSKFGI
961 KRERVPFILT YDFIHVIQGG KTGNTKFKGR FRQCCEDAYL ILRRHGNLFI TLFALMLTAG
1021 LPELTSVKDI QYLKDSLALG KSEEEALKQF KQKFDEALRE SWTTKVNWMA HTVRKDYS
  
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Recombinant p110 β nucleotide sequence:

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1  atgcatcatc accatcacca tgaattcatg tgcttcagtt tcataatgcc tcctgctatg
61  gcagacatcc ttgacatctg ggcggtggat tcacagatag catctgatgg ctccatacct
121 gtggattttc ttttggccac tgggatttat atccagttgg aggtacctcg ggaagctacc
181 atttcttata ttaagcagat gttatggaag caagttcaca attaccaat gttcaacctc
241 cttatggata ttgactccta tatgtttgca tgtgtgaatc agactgctgt atataggagg
301 cttgaagatg aaacacgaag actctgtgat gtcagacctt ttcttccagt tctcaaatta
361 gtgacaagaa gttgtgaccc aggggaaaaa ttagactcaa aaattggagt ccttatagga
421 aaaggctctg atgaatttga ttccttgaag gatcctgaag taaatgaatt tcgaagaaaa
481 atgcgcaaat tcagcgagga aaaaatcctg tcacttgtgg gattgtcttg gatggactgg
541 ctaaaacaaa catatccacc agagcatgaa ccatccatcc ctgaaaactt agaagataaa
601 ctttatgggg gaaagctcat cgtagctgtt cattttgaaa actgccagga cgtgttttagc
661 tttcaagtgt ctcctaatat gaatcctatc aaagtaaatg aattggcaat ccaaaaacgt
721 ttgactattc atgggaagga agatgaagtt agcccctatg attatgtgtt gcaagtcagc
781 gggagagtag aatatgtttt tgggtgatcat ccactaattc agttccagta tatccggaac
841 tgtgtgatga acagagccct gccccatttt atacttgtgg aatgctgcaa gatcaagaaa
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961 cctcttccat taccacaaa gaaaacacga attatttctc atgtttggga aaataacaac
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1261 aaacgaaga aatcaacgaa aactattaat ccctctaaat atcagacat caggaaagct
  
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1321 ggaaaagtgc attatcctgt agcgtgggta aatacgatgg tttttgactt taaaggacaa
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1441 atgttgaatc caatgggaac tgttcaaaca aatccatata ctgaaaatgc aacagctttg
1501 catgtttaat ttccagagaa taaaaaaca cttattatt accctccctt cgataagatt
1561 attgaaaagg cagctgagat tgcaagcagt gatagtgcata atgtgtcaag tcgaggtgga
1621 aaaaagtttc ttcctgtatt gaaagaaatc ttggacaggg atcccttgct tcaactgtgt
1681 gaaaatgaaa tggatcttat ttggactttg cgacaagact gccgagagat tttcccacaa
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P85α Sequence Information

<u>Protein</u>	Human p850α
<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:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNGYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPDLAE
121 QFAPPDIAPP LLIKLVIAIE KKGLECSLY RTQSSSNLAE LRQLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEYI QLLKKLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHG DYTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFSS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDEDLPHHD EKTWNVGSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
661 VVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNDL NVTLAYPVYA
721 QRRR

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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
121 ggattcagtg atggacagga agccaggcct gaagaaattg gctggttaaa tggctataat
181 gaaaccacag gggaaagggg ggactttccg ggaacttacg tagaatatat tggaaaggaaa
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2041 ttgcccagc cctataactt gtacagctct ctgaaagaac tggtgctaca 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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