

Certificate of Analysis

PI 3-Kinase (p110 α /p65 α)

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-790, 14-790-K, 14-790M

Parent Lot # D7EN088N

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 α and untagged, recombinant, full-length human p65 α . Co-expressed by baculovirus in Sf21 insect cells and purified using Ni²⁺/NTA-agarose.

p65 α is an oncogenic form of p85 α that binds but does not inhibit p110, leading to constitutive PI3K activity (Shekar, S.C. *et al.*, J. Biol. Chem., (2005);**280**: 27850-27855 and Jimenez, C. *et al.*, EMBO J.,(1998);**17**:743-753).

Purity (p110 α & p65 α combined) 86% by SDS-PAGE and Coomassie blue staining. p110 α MW = 125.3kDa, p65 α MW = 66.0kDa.

Specific Activity (Parent lot# D7EN088N): 596U/mg, where one unit of PI 3-Kinase alpha (p110 α /p65 α (h)) 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: 1.927mg/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.

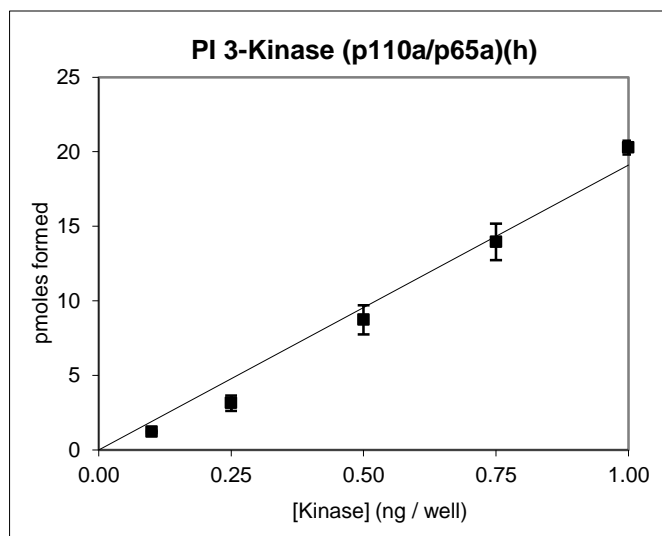
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

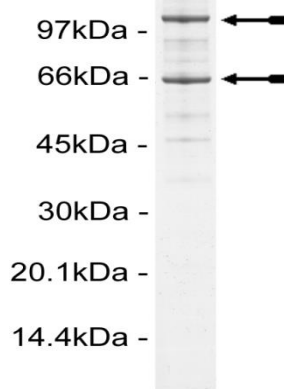
Quality Control Testing

Kinase Assay: 0.1–1ng 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 /p65 α) with the p110 α and p65 α 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 active PI 3-Kinase (p110 α /p65 α (h)).



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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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p110α Sequence Information

<u>Protein</u>	Human p110α
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human p110α
<u>Accession number</u>	GenBank U79143

Recombinant p110α amino acid sequence:

```

1  MHHHHHMP  RPSSGELWGI  HLMPPRILVE  CLLPNGMIVT  LECLREATLI  TIKHELFKEA
61  RKYPLHQLLQ  DESSYIFVSV  TQEAEREFF  DETRRLCDLR  LFQPFLKVIE  PVGNREEKIL
121  NREIGFAIGM  PVCEFDLVKD  PEVQDFRRI  LNVCKEAVDL  RDLNSPHSRA  MYVYPPNVES
181  SPELPKHIYN  KLDKGQIIVV  IWVIVSPNND  KQKYTLKINH  DCVPEQVIAE  AIRKTRSMML
241  LSSEQLKLCV  LEYQGYILK  VCGCDEYFLE  KYPLSQYKYI  RSCIMLGRMP  NLMLMAKESL
301  YSQLPMDCF  MPSYSRRIST  ATPYMNGETS  TKSLWVINS  A  LRIKILCATY  VNVNIRDIDK
361  IYVRTGIYHG  GEPLCDNVNT  QRVPCSNPRW  NEWLNYDIYI  PDLPRAARLC  LSICSVKGRK
421  GAKEEHCPLA  WGNINLFDYT  DTLVSGKMAL  NLWPVPHGLE  DLLNPIGVGT  SNPNETPCL
481  ELEFDWFSSV  VKFPDMSVIE  EHANWSVSRE  AGFSYSHAGL  SNRLARDNEL  RENDKEQLKA
541  ISTRDPLSEI  TEQEKDFLWS  HRHYCVTIPE  ILPKLLLSVK  WNSRDEVAQM  YCLVKDWPPI
601  KPEQAMELLD  CNYPDPMVRG  FAVRCLEKYL  TDDKLSQYLI  QLVQVLKYEQ  YLDNLLVRF
661  LKKALTNQRI  GHFFFHHLKS  EMHNKTVSQR  FGLLLESYCR  ACGMYLKHLN  RQVEAMEKLI
721  NLTDILKQEK  KDETQKVQMK  FLVEQMRPDP  FMDALQGF  LS  PLNPAHQ  LGN  LRLEECRIMS
781  SAKRPLWLNW  ENPDIMSELL  FQNEIIFKN  GDDLQDMLT  LQIIRIMENI  WQ  NQGLDLRM
841  LPYGCLSIGD  CVGLIEVVRN  SHTIMQIQCK  GGLKGALQFN  SHTLHQWLKD  KNKGEIYDAA
901  IDLFTRSCAG  YCVATFILGI  GDRHNSNIMV  KDDGQLFHID  FGHFLDHKKK  KFGYKRERP
961  FVL  TQDFLIV  ISKGAQECTK  TREFERFQEM  CYKAYLAIRQ  HANLFINLFS  MMLGSGMPEL
1021  QSFD  DIAYIR  KTLALDKTEQ  EALEYFMKQM  NDAHGGWTT  KMDWIFHTIK  QHALN

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Recombinant p110α nucleotide sequence:

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1  atgcatcacc  atcaccatca  catgcctcca  agaccatcat  caggtgaact  gtggggcacc  gatagtgact
61  cacttgatgc  cccaagaat  cctagtagaa  tgtttactac  caaatggaat  gatagtgact  gatagtgact
121  ttagaatgcc  tccgtgaggc  tacattaata  accataaagc  atgaactatt  taaagaagca  cgtaaagtgtt
181  agaaaatacc  cctccatca  acttcttcaa  gatgaatctt  cttacatttt  cgtaaagtgtt  cgtaaagtgtt
241  actcaagaag  cagaaaggga  agaatttttt  gatgaaacaa  gacgactttg  tgaccttcgg  tgaccttcgg
301  ctttttcaac  cttttttaa  agtaattgaa  ccagtaggca  accgtgaaga  aaagatcctc  aaagatcctc
361  aatcgagaaa  ttggttttgc  tatcggcatg  ccagtggtgt  aatttgatat  ggtaaagaat  ggtaaagaat
421  ccagaagtac  aggacttccg  aagaaatatt  ctgaacgttt  gtaaagaagc  tgtggatcct  tgtggatcct
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601  atctgggtaa  tagtttctcc  aaataatgac  aagcagaagt  atactctgaa  aatcaacct  aatcaacct
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1261  ggtgctaaag  aggaacactg  tccattggca  tggggaaata  taaactgtt  tgattacaca  tgattacaca
1321  gacactctag  tatctggaaa  aatggctttg  aatctttggc  cagtacctca  tggattagaa  tggattagaa

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1381 gatttgctga accctattgg tgttactgga tcaaatccaa ataaagaac tccatgctta
1441 gaggtagagt ttgactgggt cagcagtgtg gtaaagtcc cagatatgtc agtgattgaa
1501 gagcatgcca attggtctgt atcccagaaa gcaggattta gctattcca cgcaggactg
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3181 aaaatggatt ggatcttcca cacaattaa cagcatgcat tgaactga

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p65α Sequence Information

<u>Protein</u>	Human p65α
<u>Tags</u>	Untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human p65α
<u>Accession number</u>	GenBank XM_043865

Recombinant p65α amino acid sequence:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK KISPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDAE
121 QFAPPDIAPP LLIKLVIAIE KKGLECSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPI AVYSEMISLA PEVQSSEFYI QLLKLLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNSMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHG DYTL TLRKGGNNKL IKIFHRDGKY GFS DPLTFSS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKLLH EYNTQFQEK S REYDRLYEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL I
    
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Recombinant p65α nucleotide sequence:

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1 atgagtgctg aggggtacca gtacagagcg ctgtatgatt ataaaaagga aagagaagaa
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121 ggattcagtg atggacagga agccaggcct gaagaaattg gctggttaaa tggctataat
181 gaaaccacag gggaaagggg ggactttccg ggaacttacg tagaatatat tggaaaggaaa
241 aaaatctcgc ctcccacacc aaagccccgg ccacctcggc ctcttcctgt tgcaccaggt
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1561 atacaagga ttatgcataa ttatgataag ttgaagtctc gaatcagtga aattattgac
    
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1621 agtagaagaa gattggaaga agacttgaag aagcaggcag ctgagtatcg agaaattgac
1681 aaacgtatga acagcattaa accagacctt atctga

Reviewed and approved by site quality representative.

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