

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.

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.

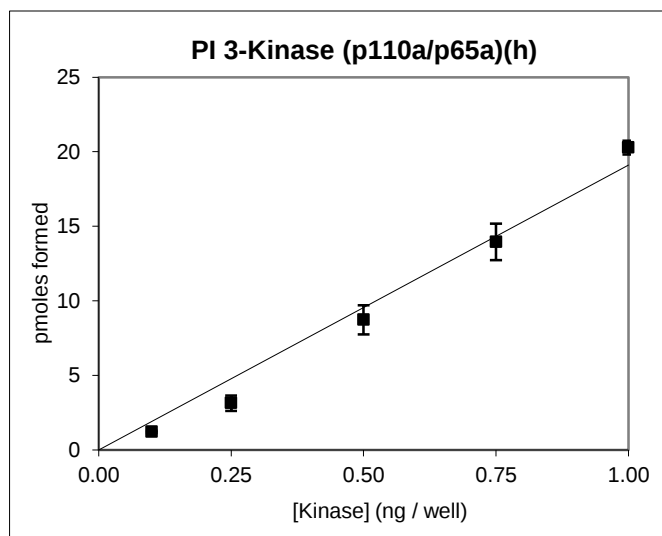
Specific Activity (Parent lot# D7EN088N): 596U/mg, where one unit of PI 3-Kinase alpha (p110 α /p65 α (h)) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate (PIP3) formed per minute at room temperature with a final ATP concentration of 100 μ M.

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**

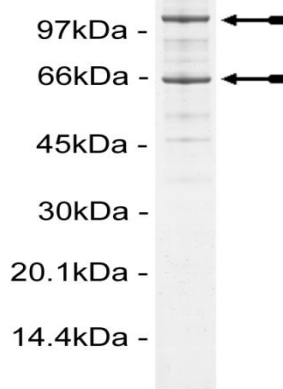
Quality Control Testing

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:

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1  MHHHHHMP RPSSGELWGI HLMPPRILVE CLLPNGMIVT LECLREATLI TIKHELFKEA
61  RKYPLHQLLQ DESSYIFVSV TQEAEREFF DETRRLCDLR LFQPFLKVIE PVGNREEKIL
121 NREIGFAIGM PVCEFDNVKD PEVQDFRRNI LNVCKEAVDL RDLNSPHSRA MYVYPPNVES
181 SPELPKHIYN KLDKGQIIVV IWVIVSPNND KQKYTLKINH DCVPEQVIAE AIRKKTRSM
241 LSSEQKLKCV LEYQGYILK VCGCDEYFLE KYPLSQYKYI RSCIMLGRMP NLMLMAKESL
301 YSQLPMDCTF MPYSRRIST ATPYMNGETS TKSLWVNSA LRIKILCATY VNVNIRDIDK
361 IYVRTGIYHG GEPLCDNVNT QRVPCSNPRW NEWLNYDIYI PDLPRAARLC LSICSVKGRK
421 GAKEEHCPLA WGNINLFDYT DTLVSGKMAL NLWPVPHGLE DLLNPIGVGTG SNPNKETPCL
481 ELEFDWFSSV VKFPDMSVIE EHANWSVSRE AGFSYSHAGL SNRLARDNEL RENDKEQLKA
541 ISTRDPLSEI TEQEKDFLWS HRHYCVTIPE ILPKLLLSVK WNSRDEVAQM YCLVKDWPPI
601 KPEQAMELLD CNYPDPMVRG FAVRCLEKYL TDDKLSQYLI QLVQVLKYEY YLDNLLVRFL
661 LKKALTNQRI GHFFFVHLKS EMHNKTVSQR FGLLLESYCR ACGMYLKHLN RQVEAMEKLI
721 NLTDILKQEK KDETQKVQMK FLVEQMRPD FMDALQGFLS PLNPAHQGLN LRLEECRIMS
781 SAKRPLWLNW ENPDIMSELL FQNNIEIFKN GDDLQDMLT LQIIRIMENI WQNGQLDLM
841 LPYGCLSIGD CVGLIEVVRN SHTIMQIQCK GGLKGALQFN SHTLHQWLKD KNKGEIYDAA
901 IDLFTRSCAG YCVATFILGI GDRHNSNIMV KDDGQLFHID FGHFLDHKKK KFGYKRERP
961 FVLTDQDFLIV ISKGAQECTK TREFERFQEM CYKAYLAIRQ HANLFINLFS MMLGSGMPEL
1021 QSFDDIAYIR KTLALDKTEQ EALEYFMKQM NDAHGGWTT KMDWIFHTIK QHALN
  
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Recombinant p110α nucleotide sequence:

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1  atgcatcacc atcaccatca catgcctcca agaccatcat caggtgaact gtggggcatc
61  cacttgatgc cccaagaat cctagtagaa tgttttactac caaatggaat gatagtgact
121 ttagaatgcc tccgtgaggc tacattaata accataaagc atgaactatt taaagaagca
181 agaaaatacc cctccatca acttcttcaa gatgaatctt cttacatttt cgtaatgtgt
241 actcaagaag cagaaaggga agaatttttt gatgaaacaa gacgactttg tgaccttcgg
301 ctttttcaac cttttttaaa agtaattgaa ccagtaggca accgtgaaga aaagatcctc
361 aatcgagaaa ttggttttgc tatcgcatg ccagtgtgtg aatttgatat ggttaaagat
421 ccagaagtac aggacttccg aagaaatatt ctgaacgttt gtaaagaagc tgtggatctt
481 agggacctca attcacctca tagtagagca atgtatgtct atcctccaaa tgtagaatct
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601 atctgggtaa tagtttctcc aaataatgac aagcagaagt atactctgaa aatcaacct
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961 gctacaccat atatgaatgg agaaacatct acaaaatccc tttgggttat aaatagtgc
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1321 gacactctag tatctggaaa aatggctttg aatctttggc cagtacctca tggattagaa
  
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1441 gagttggagt ttgactgggt cagcagtgtg gtaaagtcc cagatatgtc agtgattgaa
1501 gagcatgcca attggtctgt atcccagaaa gcaggattta gctattccca cgcaggactg
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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 EEIGWLNGYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPDLAE
121 QFAPPDIAPP LLIKLVEAIE KKGLECSLY RTQSSSNLAE LRQLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNVIPA AVYSEMISLA PEVQSSEFYI 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 I

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Recombinant p65α 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
241 aaaatctcgc ctccacaccc aaagccccgg ccacctcggc ctcttcctgt tgcaccaggt
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1501 caagagcggt acagcaaaga atacatagaa aagttttaaac gtgaaggcaa tgagaaagaa
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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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