

Certificate of Analysis

PI 3-Kinase (p110 α /p65 α), murine (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-786, 14-786-K, 14-786M

Parent Lot # D7SN037U

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 p110 α full length and untagged, recombinant, mouse p65 α full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

p65 α is an oncogenic form of p65 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 and p65 α combined) 81.5% by SDS-PAGE and Coomassie blue staining. p110 α MW = 129kDa, p65 α MW = 65.9kDa.

Specific Activity (Parent lot# D7SN037U): 611U/mg, where one unit of PI 3-Kinase (p110 α /p65 α) 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: 1.165mg/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.

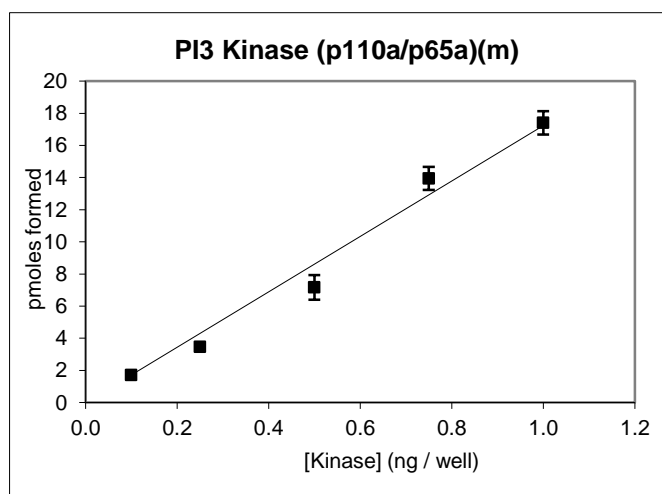
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

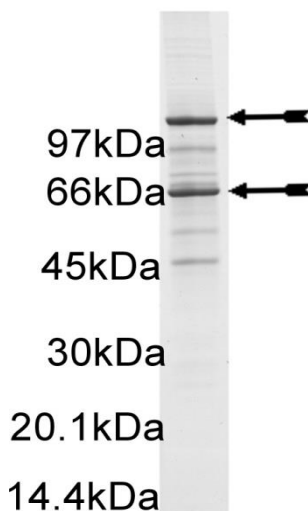
Quality Control Testing

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 sequences 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).



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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 α Sequence Information

<u>Protein</u>	Murine p110 α
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M37 of the recombinant sequence is equivalent to M1 of murine p110 α
<u>Accession number</u>	GenBank BC089038

Recombinant p110 α amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELFR EAR KYPLHQLLQD ETSYIFVSVT QEAEREFFD
121 ETRRLCDLRL FQPFLKVI EP VGNREEKILN REIGFVIGMP VCEFDMVKDP EVQDFRRNIL
181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQIIVVI WVIVSPNNDK
241 QKYTLKINH D CVPEQVIAEA IRKKTRSM LL SSEQLKLCVL EYQGYILKV CGDEYFLEK
301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
361 KSLWVINSAL RIKILCATYV NVNIRIDDKI YVRTGIYHGG EPLCDNVNTQ RVPCSNPRWN
421 EWLNYDIYIP DLPRAARLCL SICSVKGRKG AKEEHCP LAW GNINLFDYTD TLVSGKMALN
481 LWPVPHGLED LLNPIGVTGS NPNKETPCLE LEFDWFSSVV KFPDMSVIEE HANWSVSREA
541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSEIT EQEKDFLWSH RHYCVTIPEI
601 LPKLLLSVKW NSRDEVAQMY CLVKDWPPIK PEQAMELLDC NYPDPMVRSF AVRACLEKYLT
661 DDKLSQYLIQ LVQVLKYEQY LDNLLVRFLL KKALTNQRIG HFFFVHLKSE MHNKTVSQRF
721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEKK DETQKVQMKF LVEQMRQPDF
781 MDALQGFLSP LNPAHQLG NL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIIRIMENIW QNQGLDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKGALQFNS HTLHQWLKDK NKGEIYDAAI DLFTRSCAGY CVATFILGIG DRHNSNIMVK
961 DDGQLFHIDF GHFLDHK KKK FGYKRERVPF VLTQDFLIVI SKGAQEYTKT REFERFQEMC
1021 YKAYLAIRQH ANLFINLFSM MLGSGMPELQ SFDDIAYIRK TLALDKTEQE ALEYFTKQMN
1081 DAHHGGWTTK MDWIFHTIKQ HALN

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Recombinant p110 α nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggccatgga tccggaattc aaaggcctac gtcgacaaat gcctccacga
121 ccatcttcgg gtgaactgtg gggcatccac ttgatgcccc cacgaatcct agtggaatgt
181 ttactcccca atggaatgat agtgacttta gaatgcctcc gtgaggccac actcgtcacc
241 atcaaacatg aactgttcag agaggccagg aaataaccctc tccatcagct tctgcaagac
301 gaaacttctt acattttcgt aagtgtcacc caagaagcag aaagggaaga atttttgat
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421 gtaggcaacc gtgaagaaaa gatcctcaat cgagaaattg gttttgttat tggcatgcca
481 gtgtgtgaat ttgatatgg taaagatcca gaagtccaag actttcgaag gaacattctg
541 aatgtttgca aagaagctgt ggacctgagg gatctcaact cgcctcatag cagagcaatg
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1561 aagtttccag acatgtctgt gatcgaagaa catgcccaatt ggtccgtgtc ccgagaagct
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3301 catgctttga actaa
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p65α Sequence Information

Protein	Murine p65α
Tags	Untagged
Native sequence	M1 of the recombinant protein is equivalent to M1 of murine p65α
Accession number	GenBank NM_001077495

Recombinant p65α amino acid sequence:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EDIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK RISPTPKPR PPRPLPVAPG SSKTEADTEQ QALPLPDLAE
121 QFAPPDVAPP LLIKLEAIE KKGLECSTLY RTQSSSNPAE LRQLLDCDAA SVDLEMIDVH
181 VLADAFKRYL ADLNPVIPV AVYNEMMSLA QELQSPEDCI QLLKKLIRLP NIPHQCWLT
241 QYLLKHFFKL SQASSKNLLN ARVLSEIFSP VLFRFPAASS DNTEHLIKAI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN SMNNSLQD AEWYWGDISR EEVNEKLRDT ADGTFLLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDP LTFNS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVKED NIEAVGKKLH EYNTQFQEK REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL I
    
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Recombinant p65α nucleotide sequence:

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1 atgagtgctg aggggtacca gtacagagca ctgtacgact acaagaagga gcgagaggaa
61 gacattgacc tacacctggg ggacatactg actgtgaata aaggctcctt agtggcactt
121 ggattcagtg atggccagga agcccggcct gaagatattg gctgggttaa tggctacaat
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1681 aaacgatga acagtattaa gccggacctc atctaa
    
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Reviewed and approved by site quality representative.

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