

Certificate of Analysis

PI 3-Kinase (p110 α /p65 α)

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-790

Lot # 1653000 from Bulk # D7EN088N

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 (lot# 1653000): 541U/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. Calculated U/mg was determined after conversion of the HTRF ratio to pmol formed using the PIP3 calibration curve described below.

Formulation: 10 μ g of enzyme in 5.2 μ l of 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.

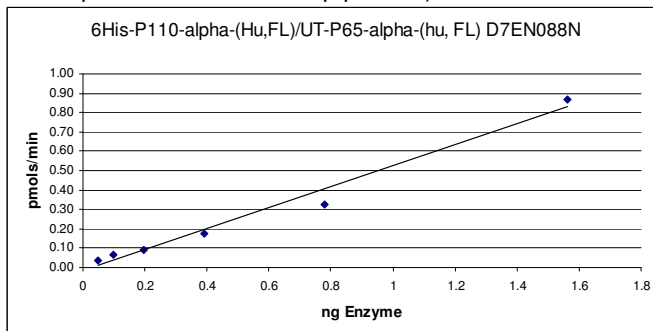
Storage and Stability: Store at -70 $^{\circ}$ C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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 $^{\circ}$ C.

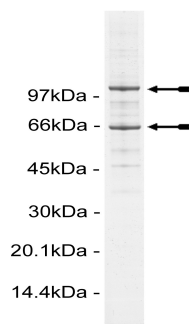
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.04–1.5ng of this lot of PI 3-kinase alpha (p110 α /p65 α (h)) was assayed in a 30 minute reaction using the PIPProfiler™ protocol (see www.upstate.com/features/pipprofiler).



MS Tryptic Fingerprint: Confirmed identity as PI 3-Kinase (p110 α /p65 α) with 48% p110 α and 55% p65 α amino acid coverage of 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 active PI 3-Kinase (p110 α /p65 α (h)).

Kinase Assay Protocol

1. Add 5µl of 10µM phosphatidylinositol 4,5-bisphosphate substrate.
2. Add 10µl (0.04–1.5ng) PI 3-Kinase (p110α/p65α (h)) active.
3. Add 5µl 100µM ATP.
4. Incubate for 30 minutes at room temperature.
5. Add 5µl Stop Buffer containing biotinylated-PIP3 and EDTA.
6. Add 5µl Detection Buffer containing Europium labelled anti-GST monoclonal antibody, GST tagged GRP1 PH domain, and Streptavidin-Allophycocyanin.
7. Read plate in HTRF mode. Product formed should be determined from a standard curve of non-biotinylated PIP3.

Generation of PIP3 calibration curve

This optional calibration should be performed on the same plate as the enzyme assay to enable appropriate conversion to pmol formed where desired.

1. Dilute phosphatidylinositol 3,4,5-trisphosphate in 1 x reaction buffer containing phosphatidylinositol 4,5-bisphosphate substrate. Start concentration curve from 10µM (final assay concentration) and dilute in semi-log steps for 12 points. Include a zero PIP3 point.
2. Add 15µl of each dilution point to appropriate wells of a black 384-well microtitre plate.
3. Add 5µl of ATP to all wells
4. Add 5µl Stop Buffer containing biotinylated-PIP3 and EDTA.
5. Add 5µl Detection Buffer containing Europium labelled anti-GST monoclonal antibody, GST tagged GRP1 PH domain, and Streptavidin-Allophycocyanin.
6. Read plate in HTRF mode and fit data to a four-parameter logistic.

p110α Sequence Information

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

Recombinant p110α amino acid sequence:

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1 MHHHHHHMPP RPSSGELWGI HLMPPRILVE CLLPNGMIVT LECLREATLI TIKHELFKEA
61 RKYPLHQLLQ DESSYIFVSV TQEAEREFF DETRRLCDLR LFQPFLLKVE PVGNREEKIL
121 NREIGFAIGM PVCFDFMVKD PEVQDFRRNI LNVCKEAVDL RDLNSPHSRA MYVYPPNVES
181 SPELPKHIYN KLDKGQIIVV IWVIVSPNND KQKYTLKINH DCVPEQVIAE AIRKKTRSML
241 LSSEQLKLCV LEYQGYILK VCGCDEYFLE KYPLSQYKYI RSCIMLGRMP NLMLMAKESL
301 YSQLPMDCFM MPYSRRIST ATPYMNGETS TKSLWVINSALRIKILCATY VNVNIRDIDK
361 IYVRTGIYHG GEPLCDNVNT QRVPCSNPRW NEWLNYDIYI PDLPRAARLC LSICSVKGRK
421 GAKEEHCPWA WGNINLFDYT DTLVSGKMAL NLWPVPHGLE DLLNP IGVGTG SNPNETPCL
481 ELEFDWFSSV VKFPDMSVIE EHANWSVSRE AGFSYSHAGL SNRLARDNEL RENDKEQLKA
541 ISTRDPLSEI TEQEKDFLWS HRHYCVTIPE ILPKLLLSVK WNSRDEVAQM YCLVKDWPP I
601 KPEQAMELLD CNYPDPMVRG FAVRCLEKYL TDDKLSQYLI QLVQVLKYEQ YLDNLLVRF L
661 LKKALTNQRI GHFFFHWLKS EMHNKTVSQR FGLLLESYCR ACGMYLKHLN RQVEAMEKLI
721 NLTDLKQEK KDETQKVQMK FLVEQMRRPD FMDALQGFLS PLNPAHQGN LRLEECRIMS
781 SAKRPLWLNW ENPDIMSELL FQNNELIFKN GDDLQDMLT LQIRIMENI WQNGGLDLRM
841 LPYGCLSIGD CVGLIEVVRN SHTIMQIQCK GGLKGALQFN SHTLHQWLKD KNGEYDAA
901 IDLFTRSCAG YCVATFILGI GDRHNSNIMV KDDGQLFHID FGHFLDHKKK KFGYKRERVP
961 FVLTQDFLIV 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 tgtttactac caaatggaat gatagtgact
121 ttagaatgcc tccgtgaggc tacattaata accataaagc atgaactatt taaagaagca
181 agaaaatacc ccctccatca acttcttcaa gatgaatctt cttacatddd cgtaagtgtt
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p65α Sequence Information

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

Recombinant p65α amino acid sequence:

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1  MSAEGYQYRA  LYDYKKEREE  DIDLHLGDIL  TVNKGSLVAL  GFSDGQEARP  EEIGWLNQYN
61  ETTGERGDFP  GTYVEYIGRK  KISPPTPKPR  PPRPLPVAPG  SSKTEADVEQ  QALTLPLDLAE
121 QFAPPDIAPP  LLIKLVEAIE  KKGLEECSTLY  RTQSSSNLAE  LRQLLDCDTP  SVDLEMIDVH
181 VLADAFKRYL  LDLPNPVIPA  AVYSEMISLA  PEVQSSEYYI  QLLKKLIRSP  SIPHQYWLTL
241 QYLLKHFFFKL  SQTSSKNLLN  ARVLSEIFSP  MLFRFSAASS  DNTENLIKVI  EILISTEWNE
301 RQPAPALPPK  PPKPTTVANN  GMNNSMSLQD  AEWYWGDISR  EEVNEKLRDT  ADGTFLVRDA
361 STKMHGDYTL  TLRKGGNNKL  IKIFHRDGKY  GFSDPLTFSS  VVELINHYRN  ESLAQYNPKL
421 DVKLLYPVSK  YQQDQVVKED  NIEAVGKKLH  EYNTQFQEK  REYDRLYEEY  TRTSQEIQMK
481 RTAIEAFNET  IKIFEEQCQT  QERYSKEYIE  KFKREGNEKE  IQRIMHNYDK  LKSRISEIID
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Recombinant p65α nucleotide sequence:

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