Certificate of Analysis

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PI 3-Kinase (p110α/p65α)

(recombinant enzyme expressed in Sf21 insect cells)
Cat # 14-790M
Lot # D7EN088N

Product Description: Complex of *N*-terminal 6His-tagged, recombinant, full-length human p110α and untagged, recombinant, full-length human p65α. Co-expressed by baculovirus in Sf21 insect cells and purified using Ni^{2+}/NTA-agarose.
p65α is an oncogenic form of p85α 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 (p110α & p65α combined) 86% by SDS-PAGE and Coomassie blue staining. p110α MW = 125.3kDa, p65α MW = 66.0kDa.

Specific Activity (Lot D7EN088N): 541U/mg, where one unit of PI 3-Kinase alpha (p110α/p65α (h)) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate (PIP3) formed per minute at room temperature with a final ATP concentration of 100µM. Calculated U/mg was determined after conversion of the HTRF ratio to pmol formed using the PIP3 calibration curve described below.

Formulation: 250µg of enzyme in **129.7µl** 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°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

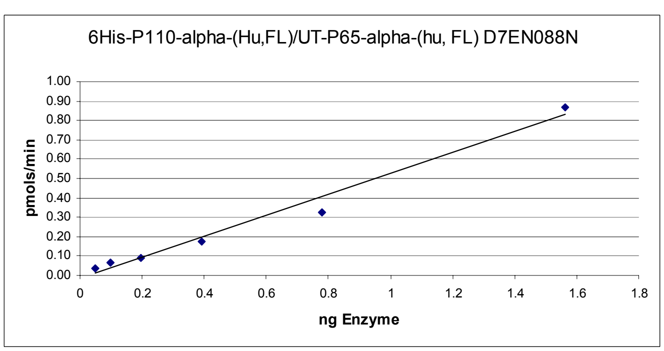
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

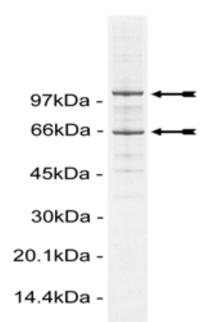
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.04 – 1.5ng of this lot of PI 3-kinase alpha (p110α/p65α (h)) was assayed in a 30 minute reaction using the PIProfiler™ protocol (see www.upstate.com/features/piprofiler).

MS Tryptic Fingerprint: Confirmed identity as PI 3-Kinase (p110α/p65α) with 48% p110α and 55% p65α amino acid coverage of the translated sequence listed on page 3.





SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active PI 3-Kinase (p110α/p65α(h))

Kinase Assay Protocol

1. Add 5µl of 60µM phosphatidylinositol 4,5-bisphosphate substrate.
2. Add 10µl (0.04 – 1.5ng) PI 3-Kinase (p110α/p65α (h)) active.
3. Add 5µl 400µM ATP.
4. Incubate for 30 minutes at room temperature.
5. Add 5 µl Stop Buffer containing biotinylated-PIP3 and EDTA.
6. Add 5 µl Detection Buffer containing Europium labeled 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 1x reaction buffer containing phosphatidylinositol 4,5-bisphoshate substrate. Start concentration curve from 10 µM (final assay concentration) and dilute in semi-log steps for 12 points. Include a zero PIP3 point.
2. Add 15 µl of each dilution point to appropriate wells of a black 384-well microtitre plate.
3. Add 5 µl of ATP to all wells
4. Add 5 µl Stop Buffer containing biotinylated-PIP3 and EDTA.
5. Add 5 µl Detection Buffer containing Europium labeled 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 tag.

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 TQEAEREEFF DETRRLCDLR LFQPFLKVIE PVGNREEKIL
 121 NREIGFAIGM PVCEFDMVKD PEVQDFRRNI LNVCKEAVDL RDLNSPHSRA MYVYPPNVES
 181 SPELPKHIYN KLDKGQIIVV IWVIVSPNND KQKYTLKINH DCVPEQVIAE AIRKKTRSML
 241 LSSEQLKLCV LEYQGKYILK VCGCDEYFLE KYPLSQYKYI RSCIMLGRMP NLMLMAKESL
 301 YSQLPMDCFT MPSYSRRIST ATPYMNGETS TKSLWVINSA LRIKILCATY VNVNIRDIDK
 361 IYVRTGIYHG GEPLCDNVNT QRVPCSNPRW NEWLNYDIYI PDLPRAARLC LSICSVKGRK
 421 GAKEEHCPLA WGNINLFDYT DTLVSGKMAL NLWPVPHGLE DLLNPIGVTG SNPNKETPCL
 481 ELEFDWFSSV VKFPDMSVIE EHANWSVSRE AGFSYSHAGL SNRLARDNEL RENDKEQLKA
 541 ISTRDPLSEI TEQEKDFLWS HRHYCVTIPE ILPKLLLSVK WNSRDEVAQM YCLVKDWPPI
 601 KPEQAMELLD CNYPDPMVRG FAVRCLEKYL TDDKLSQYLI QLVQVLKYEQ YLDNLLVRFL
 661 LKKALTNQRI GHFFFWHLKS EMHNKTVSQR FGLLLESYCR ACGMYLKHLN RQVEAMEKLI
 721 NLTDILKQEK KDETQKVQMK FLVEQMRRPD FMDALQGFLS PLNPAHQLGN LRLEECRIMS
 781 SAKRPLWLNW ENPDIMSELL FQNNEIIFKN GDDLRQDMLT LQIIRIMENI WQNQGLDLRM
 841 LPYGCLSIGD CVGLIEVVRN SHTIMQIQCK GGLKGALQFN SHTLHQWLKD KNKGEIYDAA
 901 IDLFTRSCAG YCVATFILGI GDRHNSNIMV KDDGQLFHID FGHFLDHKKK KFGYKRERVP
 961 FVLTQDFLIV ISKGAQECTK TREFERFQEM CYKAYLAIRQ HANLFINLFS MMLGSGMPEL
1021 QSFDDIAYIR KTLALDKTEQ EALEYFMKQM NDAHHGGWTT KMDWIFHTIK QHALN
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Recombinant p110α nucleotide sequence:

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 601 atctgggtaa tagtttctcc aaataatgac aagcagaagt atactctgaa aatcaaccat
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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 EEIGWLNGYN
 61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPDLAE
121 QFAPPDIAPP LLIKLVEAIE KKGLECSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEYI QLLKKLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFSS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEKS REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
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Recombinant p65α nucleotide sequence:

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