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Certificate of Analysis

PI 3-Kinase (p110 α (E545K)/p85 α)
(Recombinant enzyme expressed in Sf21 insect cells)
Catalogue # 14-783
Lot # 1626158 From # D7EN075N

Product Description: Complex of *N*-terminal 6His-tagged, recombinant, full-length human p110 α containing the mutation E545K and untagged, recombinant, full-length human p85 α . Co-expressed by baculovirus in Sf21 insect cells and purified using Ni²⁺/NTA-agarose.

The E545K substitution is a somatic mutation in p110 α that has been associated with tumours of the colon and brain. Combined *in vitro* and *in vivo* studies have shown that this mutation confers higher lipid kinase activity than wild type, and is able to induce oncogenic transformation. (Kang S. *et al.*, PNAS, (2005);**102**: 802-807 and Zhao J.J. *et al.*, PNAS, (2005);**102**:18443-18448).

Purity (p110 α p85 α combined) 76% by SDS-PAGE and Coomassie blue staining. p110 α (E545K) MW = 125.3kDa, p85 α MW = 83.7kDa.

Specific Activity (lot# 1626158): 598U/mg, where one unit of PI 3-Kinase alpha (p110 α (E545K)/p85 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 6.9 μ l of 50mM Tris/HCl pH8.0, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

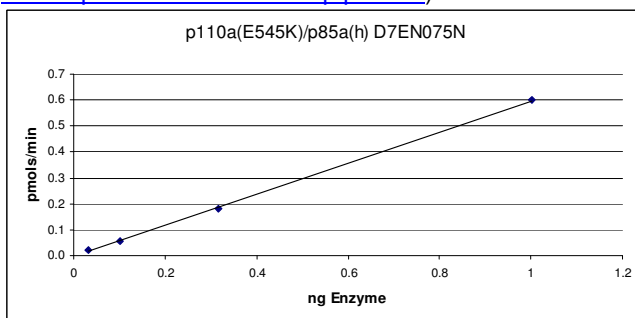
Storage and Stability: Store at -70 $^{\circ}$ C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

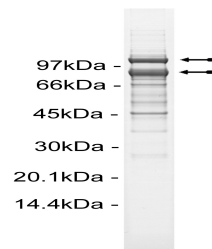
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: : 0.03 – 1 ng of this lot of PI 3-kinase alpha (p110 α (E545K)/p85 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 /p85 α) with 43% p110 α and 39% p85 α amino acid coverage of the translated sequence listed on page three.



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 (E545K)/p85 α (h)).

Kinase Assay Protocol

1. Add 5µl of 40µM phosphatidylinositol 4,5-bisphosphate substrate.
2. Add 10µl (0.03 – 1 ng) PI 3-Kinase (p110α(E545K)/p85α (h)) active.
3. Add 5µl 400µM 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 1x 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.

p110 α (E545K) Sequence Information

Protein Human p110 α (E545K)
Tags N-terminal 6His
Native sequence M8 of the recombinant protein is equivalent to M1 of human p110 α
Accession number GenBank U79143

Recombinant p110 α (E545K) amino acid sequence:

```
1 MHHHHHHMPP RPSSGELWGI HLMPPRILVE CLLPNGMIVT LECLREATLI TIKHELFKEA
61 RKYPLHQLLQ DESSYIFVSV TQEAEREIEFF DETRRLCDLR LFQPFLLKLVIE PVGNREEKIL
121 NREIGFAIGM PVCEFDMVKD PEVQDFRRNI LNVCKEAVDL RDLNSPHSRA MYVYPPNVES
181 SPELPKHIIYN KLDKGQIIIV IWVIVSPNND KQKYTLKINH DCVPEQVIAE AIRKKTRSML
241 LSSEQLKLCV LEYQGYILK VCGCDEYFLE KYPLSQYKYI RSCIMLGRMP NLMLMAKEESL
301 YSQLPMDCFM MPYSRRIST ATPYMNGETS TKSLWVINSALRIKILCATY VNVNIRDIDK
361 IYVRTGIYHG GEPLCDNVNT QRVPASNPRW NEWLNYDIYI PDLPRAARLC LSICSVKGRK
421 GAKEEHCPPLA WGNINLFDYT DTLVSGKMAL NLWPVPHGLE DLLNPIGVTG SNPNETPCL
481 ELEFDWFSSV VKFPDMSVIE EHANWSVSRE AGFSYSHAGL SNRLARDNEL RENDKEQLKA
541 ISTRDPLSEI TKQEKDFLWS HRHYCVTIPE ILPKLLLSVK WNSRDEVAQM YCLVKDWPPPI
601 KPEQAMELLD CNYPDPMVRG FAVRCLEKYL TDDKLSQYLI QLVQVLKYEY YLDNLLVRFLL
661 LKKALTNQRI GHFFFVHLKS EMHNKTVSQR FGLLLESYCR ACGMYLKHLN RQVEAMEKLI
721 NLTDILKQEK KDETQKVQMK FLVEQMRRPD FMDALQGFLLS PLNPAHQQLGN LRLEECRIMS
781 SAKRPLWLNW ENPDIMSELL FQNNIEIFKN GDDLQDMLT 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 NDAHGGWTT KMDWIFHTIK QHALN
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Recombinant p110 α (E545K) 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
241 actcaagaag cagaaaagga agaatttttt gatgaaacaa gacgactttg tgaccttcgg
301 ctttttcaac cttttttaa agtaattgaa ccagtaggca accgtgaaga aaagatcctc
361 aatcgagaaa ttggttttgc tatcggcatg ccagtggtgtg aatttgatat ggtaaagat
421 ccagaagtac aggacttccg aagaaatatt ctgaacgttt gtaaagaagc tgtggatctt
481 agggacctca attcacctca tagtagagca atgtatgtct atcctccaaa tgtagaatct
541 tcaccagaat tgccaaagca catataat aaattagata aagggcaaat aatagtggtg
601 atctgggtaa tagtttctcc aaataatgac aagcagaagt atactctgaa aatcaacct
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3181 aaaatggatt ggatcttcca cacaattaa cagcatgcat tgaactga

p85α Sequence Information

Protein Human p85α
Tags Untagged
Native sequence M1 of the recombinant protein is equivalent to M1 of human p85α
Accession number GenBank XM_043865

Recombinant p85α amino acid sequence:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDLAE
121 QFAPPDIAPP LLIKLVEAIE KKGLECSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEIYI QLLKKLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNSMSLQD AEWYWGDISR EEVNEKLRDT ADGTFIVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFSS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK SREYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKRTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDDELPHHD EKTWNVGS SNRNKAENLLRG KRDGTFIVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNSDL NVTLAYPVYA
721 QQR
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Recombinant p85α nucleotide sequence:

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1 atgagtgctg aggggtacca gtacagagcg ctgtatgatt ataaaaagga aagagaagaa
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2161 cagcagaggc gatga

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