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Certificate of Analysis

PI3 Kinase (p110 δ /p85 α)

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-604

Lot # 1652286

From bulk lot # 1619204

Product Description: Complex of N-terminal 6His-tagged recombinant, full-length, human p110 δ and untagged, recombinant, full length, human p85 α . Coexpressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 91.6% by SDS-PAGE and Coomassie blue staining. P110 δ MW = 121.6kDa, p85 α MW = 83.7kDa.

Specific Activity (lot# 1652286): 714U/mg, where one unit of PI 3-kinase (p110 δ /p85 α) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate formed per minute at 22°C with a final ATP concentration of 100 μ M.

Formulation: 10 μ g of enzyme in 5.7 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°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

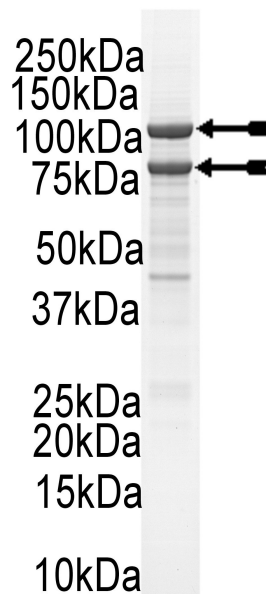
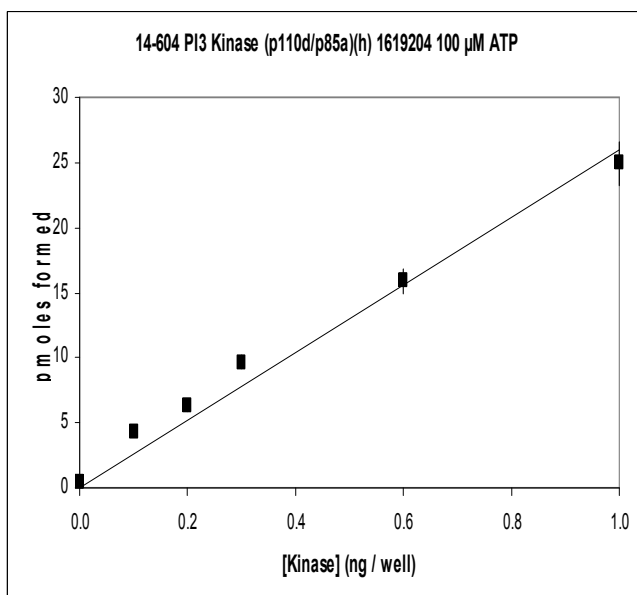
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 0.0–1.0ng of this lot of PI 3-kinase (p110 δ /p85 α) was assayed in a 30 minute reaction using the PIPProfiler™ protocol (see www.upstate.com/features/pipprofiler).

MS Tryptic Fingerprint: Confirmed product identity as p110 δ /p85 α with 32% amino acid coverage of the 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 PI3 kinase (p110 δ /p85 α).

Assay Kinase Protocol

1. Add 5µl of 10µM phosphatidylinositol 4,5-bisphosphate substrate.
2. Add 10µl (0.0–1.0ng) PI3 Kinase (p110δ/p85α) active.
3. Add 5µl 100µM ATP.
4. Incubate for 30 minutes at 22°C.
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 time resolved fluorescence mode. Product formed may be determined from a standard curve.

P1105 Sequence Information

Protein	Human p110δ
Tags	N-terminal 6His
Native sequence	M16 of the recombinant protein is equivalent to M1 of human p110δ
Accession number	GenBank NM_005026. The recombinant protein contains the amino acid substitution N253S with respect to this accession number. This conflict is reported in GenBank Y10055 and U57843. The residue coordinates in the native sequence are given.

Recombinant p110δ amino acid sequence:

```
1  MHHHHHHEFK  GLRRRMPG  V DCPMEFWTKE  ENQSVVVD  FL LPTGVYLN  FFP VSRNANLSTI
61  KQLLWHRAQY  EPLFHML  SGP EAYVFTCI  NQ TAEQQELE  DE QRRLCDV  QPF LPVLR  LVARE
121 GDRVKKLINS  QISLLIG  KGL HEFDSL  CDPE VNDFRAK  MCQ FCEEA  AARRQ QLGWE  AWLQY
181 SFPLQLEPSA  QTWGP  GTRLR PNRALL  VNVK FEGSE  ESFTF QVSTK  DVPLA LMACAL  RKKA
241 TVFRQPLVEQ  PEDYTL  QVNG RHEYLY  GSYP LCQF  QYICSC LHSGL  TPHLT MVHSS  SILAM
301 RDEQSNPAPQ  VQKPR  AKPPP IPAKK  PSSVS LWSLE  QPFRI ELIQ  GSKVNA DERMK  LVVQA
361 GLFHGNEMLC  KTVSS  SEVSV CSEP  VWKQRL EFDIN  IC DLP RMAR  LCFALY AVIEK  AKKAR
421 STKKKSKKAD  CPIAW  ANLML FDYK  DQLKTG ERCL  YMWPSV PDEK  GELLNP TGT  VRSNPNT
481 DSAAALLICL  PEVAP  HVPYY PALEK  ILELG RHSE  CVHVTE EEQ  LQLREIL ERRG  SGELYE
541 HEKDLVWKL  R HEVQ  EHFPEA LARLL  LVTKW NKHE  DVAQML YLLC  SWPELP VLSA  LELDF
601 SFPDCHVGS  F AIK  SLRKLTD DELF  QYLLQL VQVL  KYESYL DCEL  TKFLLD RALAN  RKIGH
661 FLFWHLRSEM  HVPS  VALRFG LILE  AYCRGS THH  MKVLMKQ GEAL  SKL  KAL NDFVK  LSSQK
721 TPKPQTKELM  HLCMR  QEAYL EALSH  LQSP  L DPST  LLAEVC VEQ  CTFMDSK MKPL  WIMYSN
781 EEAGSGGS  V IIF  KNGDDL R QDML  TLQMIQ LMD  VLWKQEG LDLR  MTPYGC LPT  GDRTGLI
841 EVVLRSDTIA  NIQL  NKS NMA ATA  AFNKDAL LN  WLKSKNPG EAL  DRAIEEF TL  SCAGY  CVA
901 TYVLGIGDR  H SDN  IMIRESG QLF  HIDFGHF LGN  FKTKFGI NR  ERV  PFILT YDF  VHVIQQG
961 KTNNSEK  FER FRG  YCERAY T I  LRRHGL  LFL HLF  ALMRAAG L  PEL  SCSKDI QYL  KDSLALG
1021 KTEEEAL  KH  F RVK  FNEALRE SW  KTKV  NWLA HNV  SKDN  RQ
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Recombinant p110δ nucleotide sequence:

```
1  atgcatc  atc accatc  acca tgaatt  caaaa ggcct  acgtc gacga  atgcc cctg  ggggtg
61  gactg  cccca tggaatt  ctg gacca  aaggag gaga  atcaga gcgt  tgtggt tgact  tcctg
121  ctgccc  acag gggct  ctacct gaact  tcct gtgt  cccgca atg  ccaacct cagc  accatc
181  aagcag  ctgc tgtgg  caccg cgccc  agtat gag  ccgctct tcc  acatgct cagt  ggcccc
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301  caacg  gcgtc tgtgt  gacgt gcag  ccctc ctg  cccgtcc tgc  gcctggt gg  cccgtgag
361  ggcgac  ccg tgaag  aagct cat  caactca cag  atcagcc tc  ctcatcgg ca  aaggcctc
421  cacgag  tttg actc  ctgtg cgacc  cagaa gtg  aacgact ttc  gcgccaa gat  gtgccaa
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541  agttt  ccccc tgc  agctgga gcc  ctcggt c caa  acctggg gg  cctggtac c  ctgcggctc
601  ccgaac  ccgg ccct  tctggt ca  acgttaa g ttt  gagggca gc  gaggagag ct  tcaccttc
661  caggt  gtcca cca  aggacgt gcc  gctggcg ct  gatggcct gt  gccctgcg ga  agaaggcc
721  acagt  gttcc ggc  agccgct ggt  ggagcag cc  gaagact ac  acgctgca g  gtgaacggc
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841  ctgc  acagtg gtt  gacccc tc  acctgacc at  ggtccatt c  ctctccat ct  ccgctcctg
901  cgggat  gagc agag  caacc tg  cccccag gt  ccagaaac c  gcgctgcca  accacccc
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1321 tttgactaca aggaccagct taagaccggg gaacgctgcc tctacatgtg gccctccgtc
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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:

```
1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EEIGWLNGYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDLAE
121 QFAPPDIAPP LLIKLV EAIE KKGLE CSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNVIPA AVYSEMI SLA PEVQSSE EYI 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 EYNTQFQEK S REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGV R QKKLNEWLGN
601 ENTEDQYSLV EDDDELPHHD EKTWNVGS SN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNSDL NVTLAYPVYA
721 QRR
```

Recombinant p85α nucleotide sequence:

```
1 atgagtgctg aggggtacca gtacagagcg ctgtatgatt ataaaaagga aagagaagaa
61 gatattgact tgcacttggg tgacatattg actgtgaata aagggtcctt agtagctctt
121 ggattcagtg atggacagga agccaggcct gaagaaattg gctgggttaa tggctataat
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301 tcttcgaaaa ctgaagcaga tgttgaacaa caagctttga ctctcccgga tcttgcagag
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2101 acctcccttg tgcagcaaaa cgactccctc aatgtcacac tagcctacc agtatatgca
2161 cagcagaggc gatga

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