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## Certificate of Analysis

**PI 3-Kinase (p110 $\alpha$ /p85 $\alpha$ ), mouse**  
(Recombinant enzyme expressed in Sf21 insect cells)  
Catalog # 14-785  
Lot # D7EN013AN

**Product Description:** Complex of *N*-terminal 6His-tagged, recombinant, full-length mouse p110 $\alpha$  and untagged, recombinant, full-length mouse p85 $\alpha$ . Co-expressed by baculovirus in Sf21 insect cells and purified using Ni<sup>2+</sup>/NTA-agarose.

Purity (p110 $\alpha$  & p85 $\alpha$  combined) 71% by SDS-PAGE and Coomassie blue staining. p110 $\alpha$  MW = 129kDa, p85 $\alpha$  MW = 83.6kDa.

**Specific Activity (lot# D7EN013AN):** 59U/mg, where one unit of PI 3-Kinase alpha (p110 $\alpha$ /p85 $\alpha$ ) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate (PIP3) formed per minute at room temperature with a final ATP concentration of 100 $\mu$ M. Calculated U/mg was determined after conversion of the HTRF ratio to pmol formed using the PIP3 calibration curve described below.

**Formulation:** 10 $\mu$ g of enzyme in 100 $\mu$ 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.

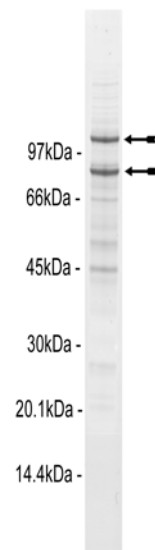
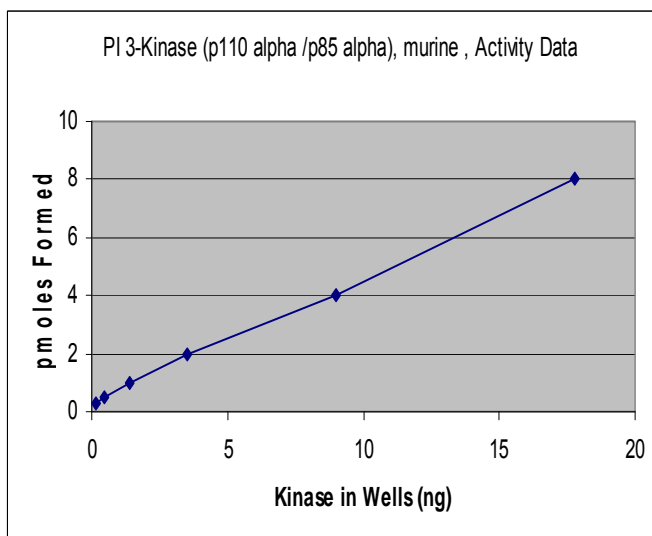
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70 $^{\circ}$ C.

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

### Quality Control Testing

**Kinase Assay:** 0.25–8ng of this lot of PI 3-kinase alpha (p110 $\alpha$ /p85 $\alpha$ ) was assayed in a 30 minute reaction using the PIPProfiler<sup>TM</sup> protocol (see [www.upstate.com/features/pipprofiler](http://www.upstate.com/features/pipprofiler)).

**MS Tryptic Fingerprint:** Confirmed identity as PI 3-Kinase (p110 $\alpha$ /p85 $\alpha$ ) with 44% p110 $\alpha$  and 39% p85 $\alpha$  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 $\mu$ g of active PI 3-Kinase (p110 $\alpha$ /p85 $\alpha$ )

## Kinase Assay Protocol

1. Add 5µl of 60µM phosphatidylinositol 4,5-bisphosphate substrate.
2. Add 10µl (0.25– 8ng) PI 3-Kinase (p110 $\alpha$ /p85 $\alpha$ ) 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 10  $\mu$ M (final assay concentration) and dilute in semi-log steps for 12 points. Include a zero PIP3 point.
2. Add 15 $\mu$ l of each dilution point to appropriate wells of a black 384-well microtitre plate.
3. Add 5 $\mu$ l 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 $\alpha$ Sequence Information

<b>Protein</b>	Mouse p110 $\alpha$
<b>Tags</b>	N-terminal 6His
<b>Native sequence</b>	M37 of the recombinant sequence is equivalent to M1 of mouse p110 $\alpha$
<b>Accession number</b>	GenBank BC089038

### Recombinant p110 $\alpha$ amino acid sequence:

```
1 MSYYHHHHHH DYDIPPTENL YFQGAMDFEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELRFREAR KYPLHQLLQD ETSYIFVSVT QEAEEREEFFD
121 ETRRLCDLRL FQPFLKVIIEP VGNREEKILN REIGFVIGMP VCEFDMVKDP EVQDFRRNIL
181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQILVVI WVIVSPNNDK
241 QKYTLKINHD CVPEQVIAEA IRKKTRSMML SSEQLKLCVL EYQGYIILKV CGCDEYFLEK
301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
361 KSLWVINSAL RIKILCATYV NVNIRDIDKI YVRTGLYHGG 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 LNPAHQGLNL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIRIMENIW QNQGLDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKGALQFNS HTLHQWLKDK NKGEIYDAAI DLFTRSCAGY CVATFILGIG DRHNSNIMVK
961 DDGQLFHIDF GHFLDHKKKK FGYKREVPVF VLTQDFLIVI SKGAQEYTKT REFERFQEMC
1021 YKAYLAIRQH ANLFINLFSM MLGSGMPELQ SFDDIAYIRK TLALDKTEQE ALEYFTKQMN
1081 DAHHGGWTTK MDWIFHTIKQ HALN
```

### Recombinant p110 $\alpha$ nucleotide sequence:

```
1 atgtcgact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gcctccacga
121 ccatcttcgg gtgaactgtg gggcatccac ttgatgcccc cacgaatcct agtggaatgt
181 ttactcccca atggaatgat agtgacttta gaatgcctcc gtgaggccac actcgtcacc
241 atcaaacatg aactgttcag agaggccagg aaataccctc tccatcagct tctgcaagac
301 gaaacttctt acattttcgt aagtgtcacc caagaagcag aaaggaaga attttttgat
361 gaaacaagac gactttgtga ccttcggcct tttcaaccct ttttaaaagt tattgaacca
421 gtaggcaacc gtgaagaaaa gatcctcaat cgagaaattg gttttgttat tggcatgcca
481 gtgtgtgaat ttgatatggg taaagatcca gaagtccaag actttcgaag gaacattctg
541 aatgtttgca aagaagctgt ggacctgagg gatctcaact cgcctcatag cagagcaatg
601 tatgtctacc ctccaaatgt cgagtcttcc ccagaactgc caaagcacat ctacaacaag
661 ttagataaag gacaaatcat agtgggtgatt tgggtaatat tctctccaaa caacgacaag
721 cagaagtaca ctctgaagat caatcatgac tgtgtgccag agcaagtcat tgctgaagcc
781 atcaggaaaa agactcggag catgtgtgtg tctctgagc agctgaaact ctgtgtctta
841 gaatatcagg gcaagtatat tctgaaagtg tgtggctgtg acgaatactt cctggaaaag
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1201 gaacccttat gtgacaatgt gaacactcaa agagtacctt gttccaatcc taggtggaat
1261 gaatggctga attatgatat atacattcct gatcttcctc gtgctgcgcg cctttgcctt
1321 tcaatctgct ctgttaaagg ccgaaagggg gctaaggagg agcactgtcc gttggcctgg
```

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1441 ctctggcctg taccgcatgg gttagaagat ctgctgaacc ctattggtgt tactgggtca  
1501 aatccaaata aagaaactcc atgcttagag ttggagtttg attggttcag cagtgtggtg  
1561 aagtttccag acatgtctgt gatcgaagaa catgcccaatt ggtccgtgtc ccgagaagct  
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1681 gaaaatgaca aggaacagct ccgagcactt tgcacccggg acccactatc tgaatcact  
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1861 tgcttagtaa aagattggcc tccaatcaaa ccagagcaag ccatggaact cctggactgt  
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2221 caagtagagg ccatggagaa gctcatcaac ctaacggaca tccttaagca ggagaagaag  
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3121 atgcttggct ctggaatgcc agaactacia tcttttgatg acattgcata tatccgaaag  
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3241 gatgcacatc atggtggatg gacgacaaaa atggattgga tcttccacac catcaagcag  
3301 catgctttga actaa

## p85 $\alpha$ Sequence Information

**Protein** Mouse p85 $\alpha$   
**Tags** Untagged  
**Native sequence** M1 of the recombinant protein is equivalent to M1 of mouse p85 $\alpha$   
**Accession number** GenBank NM\_001077495

### Recombinant p85 $\alpha$ amino acid sequence:

```
1  MSAEGYQYRA  LYDYKKEREE  DIDLHLGDIL  TVNKGSLVAL  GFSDGQEARP  EDIGWLNGYN
61  ETTGERGDFP  GTYVEYIGRK  RISPPTPKPR  PPRPLPVAPG  SSKTEADTEQ  QALPLPDLAE
121 QFAPPDVAPP  LLIKLEAIE  KKGLECSTLY  RTQSSSNPAE  LRQLLDCDAA  SVDLEMIDVH
181 VLADAFKRYL  ADLPNPVIPV  AVYNEMMSLA  QELQSPEDCI  QLLKKLIRLP  NIPHQCWLTL
241 QYLLKHFFKL  SQASSKNLLN  ARVLSEIFSP  VLFRRFPAASS  DNTEHLIKAI  EILISTEWNE
301 RQPAPALPPK  PPKPTTVANN  SMNNNMSLQD  AEWYWGDISR  EEVNEKLRDT  ADGTFLVRDA
361 STKMHG DYTL  TLRKGGNNKL  IKIFHRDGKY  GFSDPLTFNS  VVELINHYRN  ESLAQYNPKL
421 DVKLLYPVSK  YQQDQVVKED  NIEAVGKKLH  EYNTQFQEK  REYDRLYEY  TRTSQEIQMK
481 RTAIEAFNET  IKIFEEQCQT  QERYSKEYIE  KFKREGNEKE  IQRIMHNHDK  LKSRISEIID
541 SRRRLEEDLK  KQAAEYREID  KRMNSIKPDL  IQLRKTRDQY  LMWLTQKGV  R  QKKLNEWLGN
601 ENTEDQYSLV  EDDEDLPHHD  EKTWNVGSSN  RNKAENLLRG  KR DGTFLVRE  SSKQGCYACS
661 VVVDGEVKHC  VINKTATGYG  FAEPYNLYSS  LKELVLHYQH  TSLVQHNSL  NVTLAYPVYA
721  QQR
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### Recombinant p85 $\alpha$ nucleotide sequence:

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1  atgagtgctg  aggggtacca  gtacagagca  ctgtacgact  acaagaagga  gcgagaggaa
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1381 cggaatatg  atagattata  tgaggagtac  acccgactt  cccaggaaat  ccaaatgaaa
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1561 attcaaagga  ttatgcataa  ccatgataag  ctgaagtcgc  gtatcagtga  gatcattgac
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1681 aaacgcatga acagtattaa gccggacctc atccagttga gaaagacaag agaccaatac  
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2101 acctccctcg tgcagcacia tgactccctc aatgtcacac tagcataccc agtatatgca  
2161 caacagaggc gataa

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