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## Certificate of Analysis

**PI 3-Kinase (p110 $\alpha$ /p65 $\alpha$ ), murine**  
(Recombinant enzyme expressed in Sf21 insect cells)  
Catalogue # 14-786  
Lot # 1637185 From bulk lot # D7SN037U

**Product Description:** Complex of N-terminal 6His-tagged recombinant mouse p110 $\alpha$  full length and untagged, recombinant, mouse p65 $\alpha$  full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA-agarose.

p65 $\alpha$  is an oncogenic form of p65 $\alpha$  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 $\alpha$  and p65 $\alpha$  combined) 81.5% by SDS-PAGE and Coomassie blue staining. p110 $\alpha$  MW = 129kDa, p65 $\alpha$  MW = 65.9kDa.

**Specific Activity (lot# 1637185):** 1298U/mg, where one unit of PI 3-Kinase (p110 $\alpha$ /p65 $\alpha$ ) 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 8.6 $\mu$ 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.

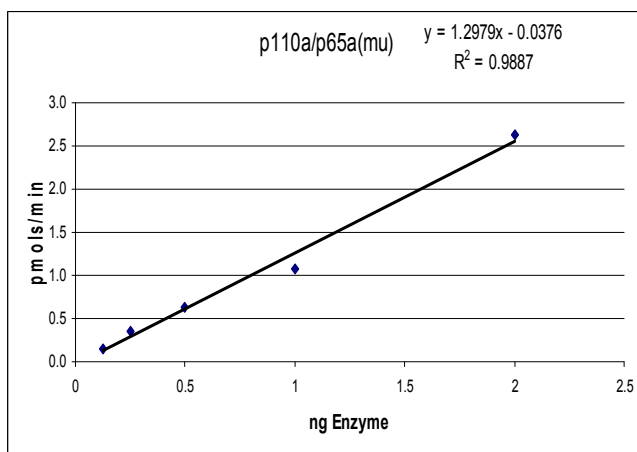
**Storage and Stability:** Store at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

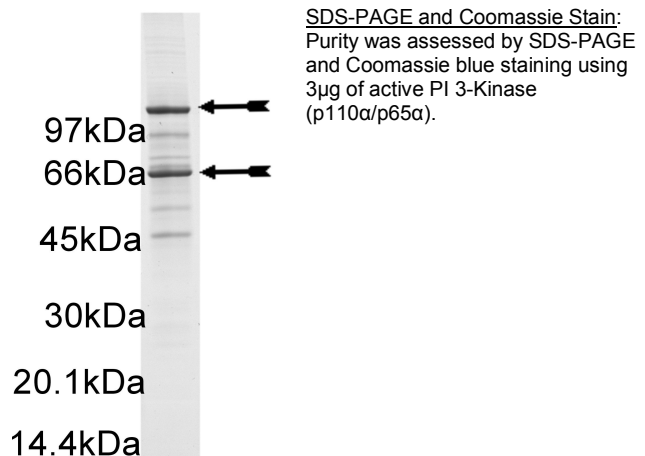
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 0.1–2.0ng of this lot of PI 3-kinase alpha (p110 $\alpha$ /p65 $\alpha$ ) was assayed in a 30 minute reaction using the PIPProfiler™ protocol described on page two.



**MS Tryptic Fingerprint:** Confirmed identity as PI 3-Kinase (p110 $\alpha$ /p65 $\alpha$ ) with 40% p110 $\alpha$  and 39% p65 $\alpha$  amino acid coverage of the translated sequences listed on pages three and five.



### Kinase Assay Protocol

Protocol for the PI 3-K HTRF™ assay (Catalogue # 33-017) found at <http://www.millipore.com/coa/tech1/74jt4z>, summarized below.

1. Add 5µl of 10µM of **phosphatidylinositol 4,5-bisphosphate (PIP2) substrate**.
2. Add **10µl (0.1–2.0ng) PI 3-Kinase (p110α/p65α)**.
3. Add 5µl of 100µM ATP.
4. Incubate for 30 minutes at room temperature.
5. Add 5µl of Stop Buffer containing biotinylated-PIP3 and EDTA.
6. Add 5µl of 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 PIP3 in 1 x Reaction Buffer containing PIP2 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 of Stop Buffer containing biotinylated-PIP3 and EDTA.
5. Add 5µl of 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** Murine p110α  
**Tags** N-terminal 6His  
**Native sequence** M37 of the recombinant sequence is equivalent to M1 of murine p110α  
**Accession number** GenBank BC089038

### Recombinant p110α amino acid sequence:

```
1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELFPREAR KYPLHQLLQD ETSYIFVSVT QEAEREFFFD
121 ETRRLCDLRL FQPFLKVIEP VGNREEKILN REIGFVIGMP VCEFDMVKDP EVQDFRRNIL
181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQIIIVI WVIVSPNNDK
241 QKYTLKINHD CVPEQVIAEA IRKKTRSMML SSEQLKLCVL EYQGGYILKV CGCDEYFLEK
301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
361 KSLWVINSAL RIKILCATYV NVNIRDIDKI YVRTGIYHGG EPLCDNVNTQ RVPCSNPRWN
421 EWLNYDIYIP DLPRAARLCL SICSVKGRKG AKEEHCPLAW GNINLFDYTD TLVSGKMALN
481 LWPVPHGLED LLNPIGV TGS NPNKETPCLE LEFDWFSSVV KFPDMSVIEE HANWSVSREA
541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSEIT EQEKDFLWSH RHYCVTIPEI
601 LPKLLLSVKW NSRDEVAQMY CLVKDWPP IK PEQAMELLDC NYPDPMVRSF AVRACLEKYL T
661 DDKLSQYLIQ LVQVLKYEQY LDNLLVRFLL KKALTNQRIG HFFFVHLKSE MHNKTVSQRF
721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEKK DETQKVQMKF LVEQMRQPDF
781 MDALQGFLSP LNP AHQLGNL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIIRIMENIW QNQGDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKGALQFNS HTLHQWLKDK NKGEIYDAAI DLFTRSCAGY CVATFILGIG DRHNSNIMVK
961 DDGQLFHIDF GHFLDHKKKK FGYKRERVPF VLTQDFLIVI SKGAQEYTKT REFERFQEMC
1021 YKAYLAIRQH ANLFINLFSM MLGSGMPELQ SFDDIAYIRK TLALDKTEQE ALEYFTKQMN
1081 DAHHGGWTTK MDWIFHTIKQ HALN
```

### Recombinant p110α nucleotide sequence:

```
1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gcctccacga
121 ccatcttcgg gtgaactgtg gggcatccac ttgatgcccc caggaatcct agtggactgt
181 ttactcccca atggaatgat agtgacttta gaatgcctcc gtgaggccac actcgtcacc
241 atcaaacatg aactgttcag agaggccagg aaataccctc tocatcagct tctgcaagac
301 gaaacttctt acattttcgt aagtgtcacc caagaagcag aaagggaga atttttgat
361 gaaacaagac gactttgtga ccttcggctt tttcaaccct ttttaaaagt tattgaacca
421 gtaggcaacc gtgaagaaaa gatcctcaat cgagaaattg gttttgttat tggcatgcca
481 gtgtgtgaat ttgatatggt taaagatcca gaagtccaag actttcgaag gaacattctg
541 aatgtttgca aagaagctgt ggacctgcyg gatctcaact cgcctcatag cagagcaatg
601 tatgtctacc ctccaaatgt cgagtcttcc ccagaactgc caaagcacat ctacaacaag
661 ttagataaag gacaaatcat agtggtgatt tgggtaatag tctctccaaa caacgacaag
721 cagaagtaca ctctgaagat caatcatgac tgtgtgccag agcaagtcat tgctgaagcc
781 atcaggaaaa agactcggag catgtttgtg tcctctgagc agctgaaact ctgtgtcta
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901 taccctctga gtcagtacaa gtacataaga agctgtataa tgctggggag gatgccaac
961 ttgatgctga tggccaaaga aagcctatac tctcagctgc cgattgatag cttcacatg
1021 cegtacatac ccaggcgcac ctccacagcc acaccctaca tgaatggaga gacatctacg
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1261 gaatggctga attatgatat atacattcct gatcttctc gtgctgcgcy cctttgcctt
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1441 ctctggcctg taccgcatgg gttagaagat ctgctgaacc ctattggtgt tactgggtca  
1501 aatccaaata aagaaactcc atgcttagag ttggagtttg attggttcag cagtgtggtg  
1561 aagtttccag acatgtctgt gatcgaagaa catgccatt ggtccgtgtc ccgagaagct  
1621 ggattcagtt actcccatac aggactgagt aacagactag ccagagacaa tgagttaaga  
1681 gaaaatgaca aggaacagct ccgagcactt tgcacccggg acccactatc tgaaatcact  
1741 gaacaagaga aagacttcct atggagccac agacactact gcgtaactat tcctgaaatc  
1801 ctacccaaat tgcttctgtc tgtcaagtgg aattccagag acgaagtggc ccagatgtac  
1861 tgcttagtaa aagattggcc tccaatcaaa ccagagcaag ccatggaact cctggactgt  
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2401 aggcttgaag agtgtcgaat tatgtcctct gcaaaaaggc cactgtgggt gaattgggag  
2461 aaccagaca tcatgtcaga gctactgttt cagaacaatg agatcatctt taaaaatggc  
2521 gacgacttac ggcaagatat gttaaccctt cagatcatcc gaatcatgga gaacatctgg  
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2641 gtgggtctca tcgaggtggt gagaaactct cacacatca tgcaaatcca gtgcaaagga  
2701 ggcctgaagg gggcgctgca gttcaacagc cacacactgc atcaatggct caaggacaag  
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3121 atgcttggct ctggaatgcc agaactacaa tcttttgatg acattgcata tatccgaaag  
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3301 catgctttga actaa

## p65α Sequence Information

<b>Protein</b>	Murine p65α
<b>Tags</b>	Untagged
<b>Native sequence</b>	M1 of the recombinant protein is equivalent to M1 of murine p65α
<b>Accession number</b>	GenBank NM_001077495

### Recombinant p65α amino acid sequence:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EDIGWLNQYN
61 ETTGERGDFP GTYVEYIGRK RISPPTPKPR PPRPLPVAPG SSKTEADTEQ QALPLPDLAE
121 QFAPPDVAPP LLIKLLLEAIE KKGLECYSTLY RTQSSSNPAE LRQLLDCDAA SVDLEMIDVH
181 VLADAFKRYL ADLPNPVIPV AVYNEMMSLA QELQSPEDCI QLLKKLIRLP NIPHQCWLTTL
241 QYLLKHFFKL SQASSKNLLN ARVLSEIFSP VLFRRFPAASS DNTEHLIKAI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN SMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDTYL TLRKGGNNKL IKIFHRDGKY GFSDFPLTFNS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK S REYDRLYE EY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERY SKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL I
```

### Recombinant p65α nucleotide sequence:

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1 atgagtgctg aggggtacca gtacagagca ctgtacgact acaagaagga gcgagaggaa
61 gacattgacc tacacctggg ggacatactg actgtgaata aaggctcctt agtggcactt
121 ggattcagtg atggccagga agcccggcct gaagatattg gctgggttaa tggctacaat
181 gaaaccactg gggagagggg agactttcca ggaacttacg ttgaatacat tggaggaaa
241 agaatttcac cccctactcc caagcctcgg ccccctcgac cgcttctctg tgctccgggt
301 tcttcaaaaa ctgaagctga cacggagcag caagcgttgc cccttctctg cctggccgag
361 cagtttgccc ctctgatgtg tgccccgcct ctcttataa agctcctgga agccattgag
421 aagaaaggac tggaatgttc gactctatac agaacacaaa gctccagcaa cctgcagaa
481 ttacgacagc ttcttgattg tgatgccgcy tcagtggact tggagatgat cgacgtacac
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901 agacagccag caccagcact gcccccaaaa ccacccaagc ccaactactgt agccaacaac
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1081 tctactaaaa tgcacggcga ttacactctt acactaagga aaggaggaaa taacaaatta
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1441 agaacggcta tcgaagcatt taatgaaacc ataaaaatat ttgaagaaca atgccaacc
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1621 agtaggagga ggttggaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac  
1681 aaacgcatga acagtattaa gccggacctc atctaa

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