



Fischer Building, Gemini Crescent, Dundee,
DD2 1SW, UK
T: +44(0) 1382 561600
F: +44(0) 1382 561601
Technical Support: (US/Canada)
T: 800 437-7500 • F: 800 437-7502
www.millipore.com

Certificate of Analysis

PI3 Kinase (p110 β)

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-603, 14-603M, 14-603-K

Lot # D8PN034U

Product Description: Complex of N-terminal 6His-tagged recombinant full-length human p110 β and untagged, recombinant, full length, human p85 α . Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 78.1% by SDS-PAGE and Coomassie blue staining. P110 β MW = 124kDa, p85 α MW = 83.7kDa.

Specific Activity (lot# D8PN034U): 430U/mg, where one unit of PI3 Kinase (p110 β) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate formed per minute at 22°C with a final ATP concentration of 10 μ M.

Formulation: 2.647mg/ml of enzyme in 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.

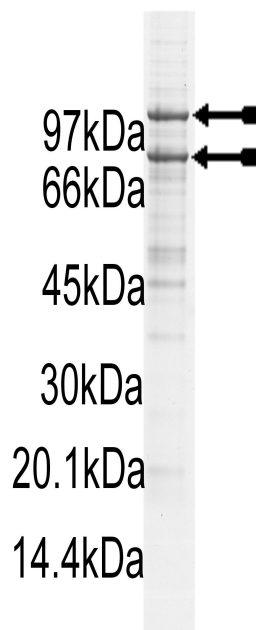
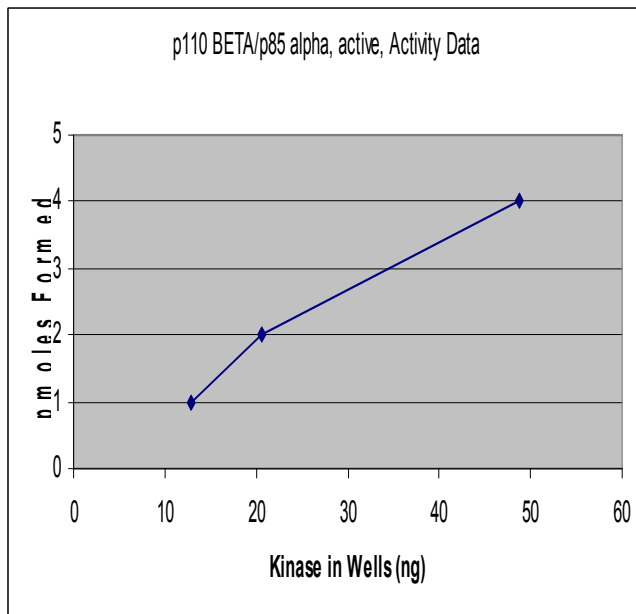
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 12.8–48.7ng of this enzyme phosphorylated 10 μ M phosphatidylinositol 4,5-bisphosphate in the assay described on page two.

MS Tryptic Fingerprint: Confirmed product identity as PI3 Kinase (p110 β) with 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 μ g of active PI3 kinase (p110 β).

Kinase Assay Protocol

1. Add 5µl of 40µM phosphatidylinositol 4, 5-bisphosphate substrate.
2. Add 10µl (12.8–48.7ng) PI3 Kinase (p110β) active.
3. Add 5µl 400µM 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.

p110 β Sequence Information

Protein Human p110 β
Tags N-terminal 6His
Native sequence M10 of the recombinant protein is equivalent to M1 of human p110 β
Accession number GenBank NM_006219

Recombinant p110 β amino acid sequence:

```
1 MHHHHHHEFM CFSFIMPPAM ADILDIWAVD SQIASDGSIP VDFLLPTGIY IQLEVPREAT
61 ISYIKQMLWK QVHNYPMFNL LMDIDSYMFA CVNQTAHYEE LEDETRRLCD VRPFLPVLKL
121 VTRSCDPGEK LDSKIGVLIG KGLHEFDSLK DPEVNEFRRK MRKFSEEKIL SLVGLSWMDW
181 LKQTYPPEHE PSIPENLEDK LYGGKLIVAV HFENCQDVFS FQVSPNMNPI KVNELAIQKR
241 LTIHGKEDDEV SPYDYVLQVS GRVEYVFGDH PLIQFYIRN CVMNRALPHF ILVECKIKK
301 MYEQEMIAIE AAINRNSSNL PLPLPPKTR IISHVWENNN PFQIVLVKGN KLNTEETVKV
361 HVRAGLFHGT ELLCKTIVSS EVSGKNDHIW NEPLEFDINI CDLPRMARLC FAVYAVLDKV
421 KTKKSTKTIN PSKYQTIRKA GKVHYPVAWV NTMVDFDKGQ LRTGDIILHS WSSFPELEE
481 MLNPMGTVQT NPYTENATAL HVKFPENKKQ PYYYPPFDKI IEKAAEIAS DSANVSSRGG
541 KKFLPVLKEI LDRDPLSQLC ENEMDLIWTL RQDCREIFPQ SLPKLLLSIK WNKLEDVAQL
601 QALLQIWPKL PPREALLELD FNYPDQYVRE YAVGCLRQMS DEELSQYLLQ LVQVLKYEPF
661 LDCALSRFLL ERALGNRRIG QFLFWHLRSE VHIPAVSVQF GVILEAYCRG SVGHMKVLSK
721 QVEALNKLKT LNSLIKLNVA KLNRAKGEA MHTCLKQSAY REALSDLQSP LNPCVILSEL
781 YVEKCKYMDS KMKPLWLIVN NKVFGEDSVG VIFKNGDDL RQDMLTLQMLR LMDLLWKEAG
841 LDLRMLPYGC LATGDRSGLI EVVSTSETIA DIQLNSSNVA AAAAFNKDAL LNWLKEYNSG
901 DDLDRAIIEF TLSCAGYCV SYVLGIGDRH SDNIMVKKTG QLFHIDFGHI LGNFKSKFGI
961 KRERVPFILT YDFIHVIQQG KTGNTKEFGR FRQCCEDAYL ILRRHGNLFI TLFALMLTAG
1021 LPELTSVKDI QYLKDSLALG KSEEEALKQF KQKFDEALRE SWTTKVNWMA HTVRKDYS
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Recombinant p110 β nucleotide sequence:

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1 atgcatcatc accatcacca tgaattcatg tgcttcagtt tcataatgcc tcctgctatg
61 gcagacatcc ttgacatctg ggcggtggat tcacagatag catctgatgg ctccatacct
121 gtggatttcc ttttgcccac tgggatttat atccagttgg aggtacctcg ggaagctacc
181 atttcttata ttaagcagat gttatggaag caagttcaca attaccaat gttcaacctc
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661 tttcaagtgt ctctaatat gaatcctatc aaagtaaatg aattggcaat ccaaaaacgt
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3181 agctggacta ctaaagtga ctggatggcc cacacagttc ggaaagacta cagatcttaa

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 EEIGWLNGYN
61 ETTGERGDFP GTYVEYIGRK KISPPTPKPR PPRPLPVAPG SSKTEADVEQ QALTLPLDLAE
121 QFAPPDIAPP LLIKLVIAIE KKGLECSTLY RTQSSSNLAE LRQLLDCDTP SVDLEMIDVH
181 VLADAFKRYL LDLPNPVIPA AVYSEMISLA PEVQSSEEIYI QLLKKLIRSP SIPHQYWLTL
241 QYLLKHFFKL SQTSSKNLLN ARVLSEIFSP MLFRFSAASS DNTENLIKVI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN GMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFSS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK S REYDRLYEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNYDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDEDLPHHD EKTWNVGSSN RNKAENLLRG KRDTGFLVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNDL NVTLAYPVYA
721 QQRR
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Recombinant p85α nucleotide sequence:

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121 ggattcagtg atggacagga agccaggcct gaagaaattg gctggttaaa tggctataat
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2161 cagcagagggc gatga

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