

Certificate of Analysis

PI 3-Kinase (p110 β /p85 α) mouse

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-794, 14-794-K, 14-794M

Parent Lot # 1606097

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

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

Purity (p110 β and p85 α combined) 80.7% by SDS-PAGE and Coomassie blue staining. p110 β MW = 126kDa, p85 α MW = 84kDa.

Specific Activity (Parent lot# 1606097): 508U/mg, where one unit of PI 3-Kinase (p110 β /p85 α) 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.

Formulation: 0.893mg/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.

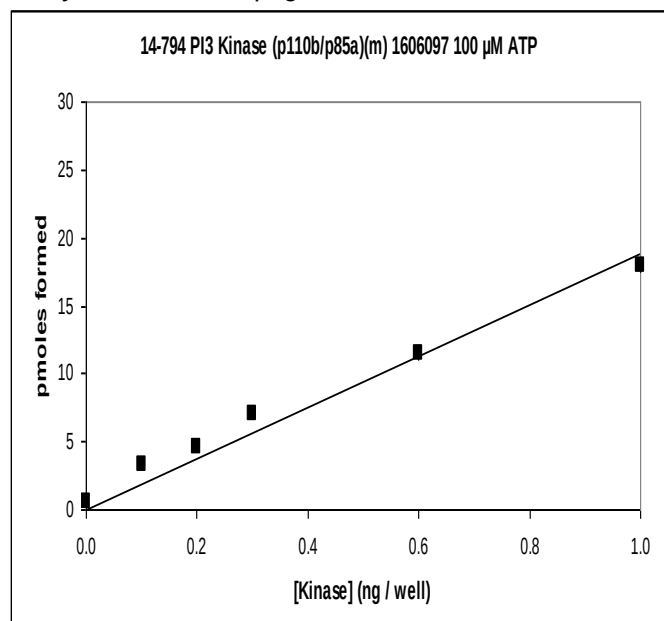
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge 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: 0–1ng of this enzyme phosphorylated 10 μ M phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.



MS Tryptic Fingerprint: Confirmed product identity as PI 3-Kinase (p110 β /p85 α) with the p110 β and p85 α amino acid translated sequences listed on pages three and five.



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Eurofins PI3 Kinase Homogeneous Time-resolved Fluorescence (HTRF) Class I Reagent Kits

The following Eurofins kits are suitable for use with this enzyme:

Cat. No	Kit Description
33-016	PI3 Kinase 4-Step Assay Reagent 1-Plate Kit
33-017	PI3 Kinase 4-Step Assay Reagent 5-Plate Kit
33-036	PI3 Kinase 4-Step Assay Reagent Kit (10000 wells)
33-037	PI3 Kinase 4-Step Assay Reagent Kit (50000 wells)
33-040	PI3 Kinase 3-Step Assay Reagent Kit (384 wells)
33-041	PI3 Kinase 3-Step Assay Reagent Kit (1920 wells)
33-047	PI3 Kinase 3-Step Assay Reagent Kit (10000 wells)

Kits 33-016, 33-017, 33-036 and 33-037 provide reagents and assay details for the Eurofins standard 4-step HTRF assay. This assay format is suitable for the majority of small and medium throughput screening work. The 3-step HTRF assay (kits 33-040, 33-041, 33-047) was introduced to reduce the number of assay steps to aid high throughput screening. Items 33-040 and 33-041 are intended as introductory kits for 3-step procedure work up. Please contact us for any further information regarding different kit formats (discoveryservices@eurofins.com).

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p110 β Sequence Information

Protein	mouse p110 β
Tags	N-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of mouse p110 β
Accession number	GenBank NM_029094. The recombinant protein contains the amino acid substitution R123L with reference to GenBank NM_029094. R123L is reported in GenBank CV558291, CO431848, CN701015, CN681036, CN536135 and CN534564.

Recombinant p110 β amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MPPAMADNLD IWAVD SQIAS DG AISVD FLL
61 PTGIYIQLEV PREATISYIK QMLWKQVHNY PMFNLLMDID SYMFACVNQT AVYEELEDET
121 RRLCDVRPFL PVLKLVTRSC DPAEKLD SKI GVLIGKGLHE FDALKDPEVN EFRRKMRKFS
181 EAKIQSLVGL SWIDWLKHTY PPEHEPSVLE NLEDKLYGGK LVVAVHFENS QDVFSFQVSP
241 NLNPIKINEL AIQKRLTIRG KEDEASPCDY VLQVSGRVEY VFGDHPLIQF QYIRNCVMNR
301 TLPHFILVEC CKIKKMYEQE MIAIEAAINR NSSNLPLPLP PKKTRVISHI WDNNNPFQIT
361 LVKGNKLNTE ETVKVHVRAG LFHGT ELLCK TVVSSEISGK NDHIWNEQLE FDINICDLPR
421 MARLCFAVYA VLDKVKTKKS TKTINPSKYQ TIRKAGKVHY PVAWVNTMVF DFKGQLRSGD
481 VILHSWSSFP DELEMLNPM GTVQTNPYAE NATALHITFP ENKKQPCYYP PFDKIIKAA
541 ELASGDSANV SSRGGKKFLA VLKEILDRDP LSQLCENEMD LIWTLRQDCR ENFPQSLPKL
601 LLSIKWNKLE DVAQLQALLQ IWPKLPPREA LELLD FNYPD QYVREYAVGC LRQMSDEELS
661 QYLLQLVQVL KYEPFLDCAL SRFLLERALD NRRIGQFLFW HLRSEVHTPA VSVQFGVILE
721 AYCRGSGVGHM KVL SKQVEAL NKLKTLNSLI KLN AVKLSRA KGKEAMHTCL KQSAYREALS
781 DLQSP LNPCV ILSELYVEKC KYMDSKMKPL WLVYSSRAFG EDSVGVIFKN GDDL RQDMLT
841 LQMLRLMDLL WKEAGLDLRM LPYGC LATGD RSG LIEVVST SETIADIQLN SSNVAATAAF
901 NKDALLNLWK EYNSGDDLDR AIEEFTLSCA GYCVASYVLG IGD R HSDNIM VKKTGQLFHI
961 DFGHILGNFK SKFGIKRERV PFILTYDFIH VIQQGKTGNT EKFGFRQCC EDAYLILRRH
1021 GNLFITLFAL MLTAGLPELT SVKDIQYLKD SLALGKSEE ALKQFKQKFD EALRESWTTK
1081 VNWMAHTVRK DYRS

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Recombinant p110 β nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atgcctcctg ctatggcaga caaccttgac
121 atctgggcag tggactcaca gattgcatcc gatggcgcca tatccgtcga tttccttctg
181 cccaccggga tttatatcca gttggaagta cctcgggaag ctaccatttc ttatatataa
241 cagatgttat ggaagcaagt tcacaactac ccgatgttta acctcctcat ggacattgac
301 tcgtatatgt ttgcatgtgt gaatcaaaact gctgtatatg aggaactgga agacgaaaca
361 cgaagacttt gtgatgtcag accttttctt ccagttctca aactagtgac tagaagctgt
421 gaccccgtag aaaaattgga ctcaaaaatt ggggttctta taggaaaagg tcttcattgag
481 tttgatgcct tgaaggatcc cgaagtgaat gaatttagaa gaaaaatgag caaattcagt
541 gaggccaaga ttcagtctct ggtaggggtg tcttggatcg actggctaaa gcacacgtat
601 ccgctgagc acgagccgtc cgtcctggag aacttgggaag ataaacttta tggaggaaag
661 ctggttgggg ctgtgcactt tgaaaatagc caggatgtat ttagttttca agtgctctcc
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901 accctgcccc acttcattct tgtggaatgt tgtaagatca agaaaatgta tgaacaagaa
961 atgattgcca tagaggctgc catcaaccga aactcatcca accttcctct ccctttacca
1021 ccaaagaaaa cgcgagttat ttctcatatc tgggacaaca acaacccttt ccaaattacc
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1321 acaaagacta ttaatccctc taagtatcag accatcagga aagccgggaa agtgcattat
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1501 gggactgtgc agacgaaccc atatgctgag aacgccaccg ccttgcacat tacgttccca
1561 gagaataaga agcagccgtg ttattatccc ccttcgata agatcattga gaaggcagct
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2101 catcttaggt cagaggtgca cactcctgct gtgtccgtac agtttgggtg catcctggaa
2161 gcatactgtc gaggaagcgt ggggcacatg aaagtgcctt ccaaacaggt ggaagcactc
2221 aataagttaa aaactttaaa tagcttaatc aaactgaatg cgggtgaagct gagcagagct
2281 aagggaaagg aggccatgca cacgtgcctg aaacagagtg cttaccggga ggcgctctct
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2761 gcgattgagg agtttacctt gtcctgtgct ggctactgtg tagcctctta tgtcctcggc
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3121 tcgggtcaaag atatacagta tcttaaggac tcgcttgctt tagggaagag cgaggaggaa
3181 gcaactgaagc agttcaagca gaagtttgac gaggccctca gggaaagctg gactactaaa
3241 gtgaactgga tggctcacac agtacggaaa gactacaggt cctaa

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p85α Sequence Information

<u>Protein</u>	mouse p85α
<u>Tags</u>	untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of mouse p85α
<u>Accession number</u>	GenBank NM_001077495

Recombinant p85α amino acid sequence:

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1  MSAEGYQYRA  LYDYKKEREE  DIDLHLGDIL  TVNKGSLVAL  GFSDGQEARP  EDIGWLNGYN
61  ETTGERGDFP  GTYVEYIGRK  RISPTPKPR  PPRPLPVAPG  SSKTEADTEQ  QALPLPDLAE
121 QFAPPDVAPP  LLIKLLEAIE  KKGLECSLY  RTQSSSNPAE  LRQLDCDAA  SVDLEMIDVH
181 VLADAFKRYL  ADLPNPVIPV  AVYNEMMSLA  QELQSPEDCI  QLLKKLIRLP  NIPHQCWLTL
241 QYLLKHFFKL  SQASSKNLLN  ARVLSEIFSP  VLFRFPAASS  DNTEHLIKAI  EILISTEWNE
301 RQPAPALPPK  PPKPTTVANN  SMNNNMSLQD  AEWYWGDISR  EEVNEKLRDT  ADGTFLVRDA
361 STKMHGDYTL  TLRKGGNNKL  IKIFHRDGKY  GFSDPLTFNS  VVELINHYRN  ESLAQYNPKL
421 DVKLLYPVSK  YQQDQVVKED  NIEAVGKKLH  EYNTQFQEK  REYDRLYEEY  TRTSQEIQMK
481 RTAIEAFNET  IKIFEEQCQT  QERYSKEYIE  KFKREGNEKE  IQRIMHNHDK  LKSRISEIID
541 SRRRLEEDLK  KQAAEYREID  KRMNSIKPDL  IQLRKTRDQY  LMWLTQKGVR  QKKLNEWLGN
601 ENTEDQYSLV  EDDELPHHD  EKTWNVGSSN  RNKAENLLRG  KRDTGFLVRE  SSKQGCYACS
661 VVDGEVKHC  VINKTATGYG  FAEPYNLYSS  LKELVLHYQH  TSLVQHNSDL  NVTLAYPVYA
721  QQR

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Recombinant p85α nucleotide sequence:

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1  atgagtgtctg  aggggtacca  gtacagagca  ctgtacgact  acaagaagga  gcgagaggaa
61  gacattgacc  tacacctggg  ggacatactg  actgtgaata  aaggctcctt  agtggcactt
121 ggattcagtg  atggccagga  agcccgccct  gaagatattg  gctggttaaa  tggctacaat
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1321 aatattgaag  ctgtagggaa  aaaattacat  gaataataa  ctcaatttca  agaaaaaagt
1381 cggaatatg  atagattata  tgaggagtac  acccgtaact  cccaggaaat  ccaaatgaaa
1441 agaacggcta  tcgaagcatt  taatgaacc  ataaaaatat  ttgaagaaca  atgccaacc
1501 caggagcggg  acagcaaaga  atacatagag  aagtttaaac  gcgaaggcaa  cgagaaagaa
1561 attcaaagga  ttatgcataa  ccatgataag  ctgaagtcgc  gtatcagtga  gatcattgac

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1621 agtaggagga ggttgaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac
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1741 ttgatgtggc tgacgcagaa aggtgtgcgg cagaagaagc tgaacgagtg gctggggaat
1801 gaaaataccg aagatcaata ctccctggta gaagatgatg aggatttgcc ccaccatgac
1861 gagaagacgt ggaatgtcgg aagcagcaac cgaaacaaag cggagaacct attgcgaggg
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2041 ttgcccagc cctacaacct gtacagctcc ctgaaggagc tggtgctaca ttatcaacac
2101 acctccctcg tgcagcacia tgactccctc aatgtcacac tagcataccc agtatatgca
2161 caacagaggc gataa

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Reviewed and approved by site quality representative.

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