

Certificate of Analysis

PI 3-Kinase (p110β/p85α) 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 p110β, full length and untagged, recombinant, mouse p85α, full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

Purity (p110β and p85α combined) 80.7% by SDS-PAGE and Coomassie blue staining. p110β MW = 126kDa, p85α MW = 84kDa.

Specific Activity (Parent lot# 1606097): 508U/mg, where one unit of PI 3-Kinase (p110β/p85α) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate (PIP3) formed per minute at room temperature with a final ATP concentration of 100μM.

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.

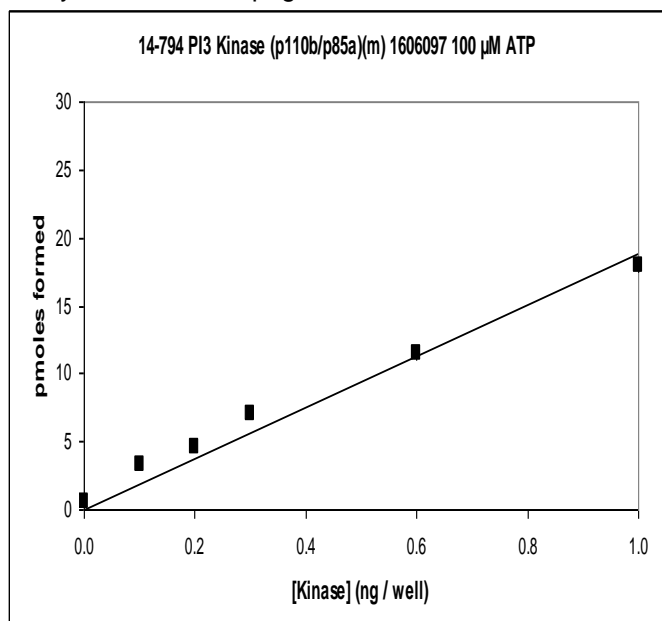
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

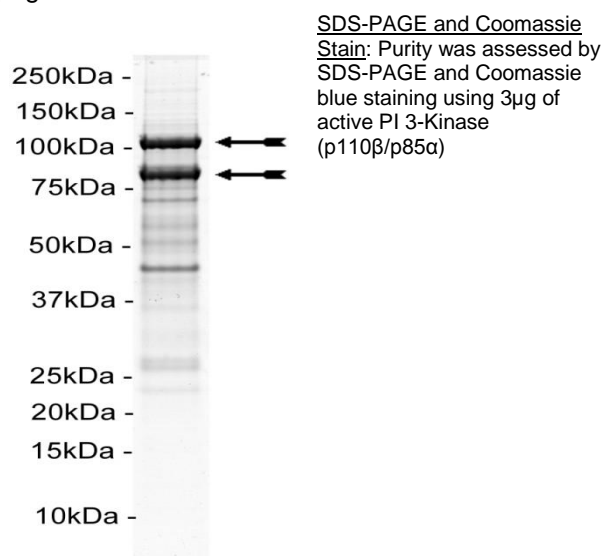
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0–1ng of this enzyme phosphorylated 10μM phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.



MS Tryptic Fingerprint: Confirmed product identity as PI 3-Kinase (p110β/p85α) with the p110β and p85α amino acid translated sequences listed on pages three and five.



Certificate of Analysis

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).

Certificate of Analysis

p110 β Sequence Information

<u>Protein</u>	mouse p110 β
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of mouse p110 β
<u>Accession number</u>	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 IWAVDSQIAS DGAISVDVFL
61 PTGIYIQLEV PREATISYIK QMLWKQVHNY PMFNLLMDID SYMFACVNQT AVYEELEDET
121 RRLCDVRPFL PVLKLVTRSC DPAEKLDISKI 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 LFHGTPELLCK TVVSSEISGK NDHIWNEQLE FDINICDLPR
421 MARLCFAVYA VLDKVKTKKS TKTINPSKYQ TIRKAGKVHY PVAWVNTMVF DFKGQLRSGD
481 VILHSWSSFP DELEMLNPM GTVQTNPYAE NATALHITFP ENKKQPCYYP PFDKIEKAA
541 ELASGDSANV SSRGGKFLA VLKEILDRDP LSQLCENEMD LIWTLRQDCR ENFPQSLPKL
601 LLSIKWNKLE DVAQLQALLQ IWPKLPPREA LELLDNFNYPD QYVREYAVGC LRQMSDEELS
661 QYLLQLVQVL KYEPFLDCAL SRFLLERALD NRRIGQFLFW HLRSEVHTPA VSVQFGVILE
721 AYCRGVSVMH KVLKQVEAL NKLKTLNSLI KLNAVKLSRA KGKEAMHTCL KQSAYREALS
781 DLQSPNPNVC ILSELYVEKC KYMDSKMKPL WLVSRAFG EDSVGVIFKN GDDLQDMLT
841 LQMLRLMDLL WKEAGLDRM LPYGCLATGD RSGLIEVVST SETIADIQLN SSNVAATAAF
901 NKDALLNWLK EYNSGDDLDR AIEEFTLSCA GYCVASYVLG IGDHSDNIM VKKTGQLFHI
961 DFGHILGNFK SKFGIKRERV PFILTYDFIH VIQQGKTGNT EKFGFRQCC EDAYLILRRH
1021 GNLFITLFA LMLTAGLPELT SVKDIQYLKD SLALGKSEEE 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 ggcctatgga tccggaattc atgcctcctg ctatggcaga caaccttgac
121 atctgggcag tggactcaca gattgatcc gatggcgcca tatccgtcga tttccttctg
181 cccaccggga tttatatcca gttggaagta cctcgggaag ctaccatttc ttatattaa
241 cagatgttat ggaagcaagt tcacaactac ccgatgttta acctcctcat ggacattgac
301 tcgtatatgt ttgcatgtgt gaatcaact gctgtatatg aggaactgga agacgaaaca
361 cgaagacttt gtgatgtcag accttttctt ccagtttctca aactagttag tagaagctgt
421 gaccccgtag aaaaattgga ctcaaaaatt ggggttctta taggaaaagg tcttcatgag
481 tttgatgcct tgaaggatcc cgaagtgaat gaatttagaa gaaaaatgcg caaattcagt
541 gaggccaaga ttcagtctct ggtagggttg tcttggatcg actggctaaa gcacacgtat
601 ccgctgagc acgagccgtc cgtcctggag aacttgggaag ataaacttta tggaggaaag
661 ctggttggg ctgtgcactt tgaaaatagc caggatgtat ttagttttca agtgtctccc
721 aatttgaatc ctataaaaat aatgaattg gcaatccaga aacgcctcac tattcgtgga
781 aaggaagatg aagctagccc ctgtgactat gtgttacagg tcagtgggag agtggagtat
841 gtgtttggcg atcatccact aattcagttc cagtacatcc ggaattgtgt gatgaataga
901 accctgccc acttcatcct tgtggaatgt tgtaagatca agaaaatgta tgaacaagaa
961 atgattgcca tagaggctgc catcaaccga aactcatcca accttctct cctttacca
1021 ccaagaaaaa cgcgagttat ttctcatatc tgggacaaca acaaccctt ccaaattacc
1081 ttggttaaag gaaataagct taatacagaa gaaactgtga aagttcatgt ccgagctggg

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1141 ctttttcacg gaaccgagct cctgtgtaaa accgtcgtaa gctcagagat atcaggaaag
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1261 atggctcgat tatgttttgc tgtttatgca gttttggata aagtaaaaac gaagaaatca
1321 acaaagacta ttaatccctc taagtatcag accatcagga aagccgggaa agtgcattat
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1441 gtcatattgc atagctggtc ttcgtttcct gatgagctgg aagaaatgct gaatccccatg
1501 gggactgtgc agacgaacc atatgctgag aacgccaccg cttgacacat tacgttccca
1561 gagaataaga agcagccgtg ttattatccc cccttcgata agatcattga gaaggcagct
1621 gagcttgcca gcggagacag tgctaagtgt tcaagtcgtg gtggaaaaaa atttcttctg
1681 gtgctgaaag aaatcttggc cagggacccc ctgtctcagc tgtgtgagaa cgaatggac
1741 cttatttggc cctacggca agactgccga gaaaatttcc ctcagtcact gccaaaacta
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1921 cagtatgtcc gggaaatcgc tgtaggctgc cttcgacaga tgagtgatga agaactctct
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2221 aataagttaa aaactttaaa tagcttaatc aaactgaatg cggtgaaagc gagcagagct
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2701 aacaaagacg cactcctgaa ctggctcaag gagtacaact ctggggatga cctggaccga
2761 gcgattgagg agtttacctt gtcctgtgct ggctactgtg tagcctctta tgcctcggc
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2881 gattttgggc atattcttgg aaatttcaa tctaaatttg gcattaaaag ggagcgagta
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3241 gtgaactgga tggctcacac agtacggaaa gactacaggt cctaa
    
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Certificate of Analysis

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 KKGLECSTLY RTQSSSNPAE LRQLLDCDAA SVDLEMIDVH
181 VLADAFKRYL ADLPNPVIPV AVYNEMMSLA QELQSPEDCI QLLKKLIRLP NIPHQCWLTL
241 QYLLKHFFKL SQASSKNLLN ARVLSEIFSP VLFRFPAASS DNTEHLIKAI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN SMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHG DYTL TLRKGGNKL IKIFHRDGKY GFS DPLTFNS VVELINHYRN ESLAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK S REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDEDLPHHD EKTWNV GSSN RNKAENLLRG KR DGTFLVRE SSKQGCYACS
661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNSL NVTLAYPVYA
721 QRRR
    
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Recombinant p85α nucleotide sequence:

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1 atgagtgctg aggggtacca gtacagagca ctgtacgact acaagaagga gcgagaggaa
61 gacattgacc tacacctggg ggacatactg actgtgaata aaggctcctt agtggcactt
121 ggattcagtg atggccagga agccccgctt gaagatattg gctggttaaa tggctacaat
181 gaaaccactg gggagagggg agactttcca ggaacttacg ttgaatacat tggaaaggaaa
241 agaatttcac cccctactcc caagcctcgg cccccctgac cgcttcctgt tgctccgggt
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421 aagaaaggac tggaaatggt gactctatac agaacacaaa gctccagcaa cctgcagaa
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1441 agaacggcta tcgaagcatt taatgaaacc ataaaaatat ttgaagaaca atgccaacc
1501 caggagcggg acagcaaaga atacatagag aagttaaacc gcgaaggcaa cgagaaagaa
1561 attcaagga ttatgcataa ccatgataag ctgaagtcgc gtatcagtga gatcattgac
    
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1621 agtaggagga ggttgaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac
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2041 tttgccgagc cctacaacct gtacagctcc ctgaaggagc tggtgctaca ttatcaacac
2101 acctccctcg tgcagcacia tgactccctc aatgtcacac tagcatatcc agtatatgca
2161 caacagaggc gataa
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Reviewed and approved by site quality representative.

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