

Certificate of Analysis

PI 3-Kinase (p110 β /p85 β), murine (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-788, 14-788-K, 14-788M

Parent Lot # D7EN092N

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 β full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity (p110 β and p85 β combined) 95% by SDS-PAGE and Coomassie blue staining. p110 β MW = 125.5kDa, p85 β MW = 81.4kDa.

Specific Activity (Parent lot# D7EN092N): 535U/mg, where one unit of PI 3-Kinase (p110 β /p85 β) 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: 2.22mg/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.

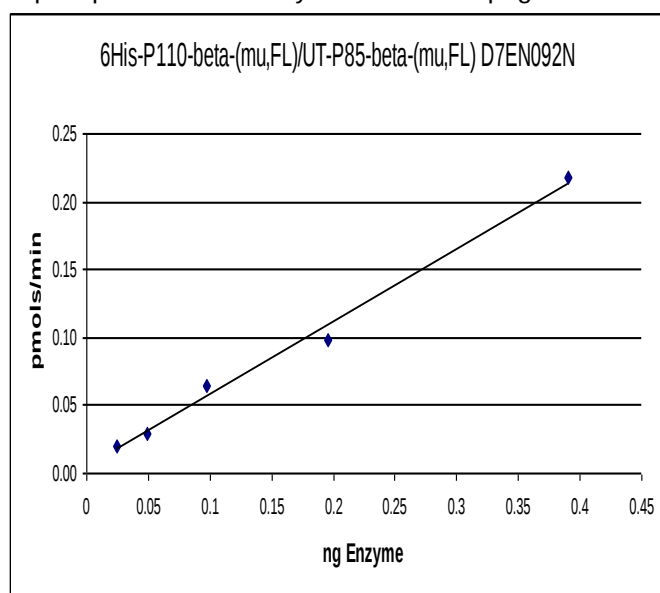
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

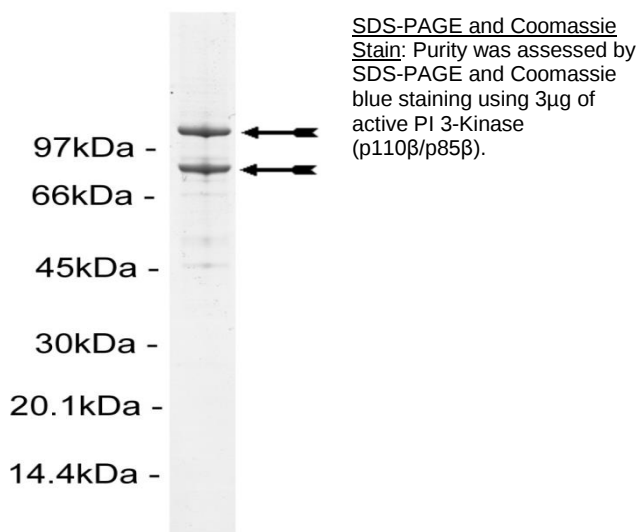
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.02–0.4ng of this enzyme phosphorylated 10 μ M phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.



MS Tryptic Fingerprint: Confirmed identity as PI 3-Kinase (p110 β /p85 β) with the p110 β and p85 β 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

<u>Protein</u>	Murine p110 β
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of murine p110 β
<u>Accession number</u>	GenBank NM_029094. The recombinant protein contains the amino acid substitution R123L (native protein coordinates) with respect to GenBank NM_029094, this is reported in the ESTs CV558291, CO431848, CN701015, CN681036, CN536135 and CN534564.

Recombinant p110 β amino acid sequence:

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1 MSYYHHHHHH 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 NLEDKLYGK LVVAVHFENS QDVFSFQVSP
241 NLNPIKINEL AIQKRLTIRG KEDEASPCDY VLQVSGRVEY VFGDHPLIQF QYIRNCVMNR
301 TLPHFILVEC CKIKKMYEQE MIAIEAAINR NSSNLPLPLP PKKTRVISHI WDNNNPFQIT
361 LVKGNKLNTE ETVKVHVVRAG LFHGT ELLCK TVVSSEISGK NDHIWNEQLE FDINICDLPR
421 MARLCFAVYA VLDKVKTKKS TKTINPSKYQ TIRKAGKVHY PVAWNTMVF DFKGQLRSGD
481 VILHSWSSFP DELEMLNPM GTVQTNPYAE NATALHITFP ENKKQPCYYP PFDKIIEKAA
541 ELASGDSANV SSRGGKKFLA VLKEILDRDP LSQLCENEMD LIWTLRQDCR ENFPQSLPKL
601 LLSIKWNKLE DVAQLQALLQ IWPKLPPREA LELLD FNYPD QYVREYAVGC LRQMSDEELS
661 QYLLQLVQVL KYEPFLDCAL SRFL LERALD NRRIGQFLFW HLRSEVHTPA VSVQFGVILE
721 AYCRG SVGHM KVL SKQVEAL NKLKTLNSLI KLN AVKLSRA KGKEAMHTCL KQSAYREALS
781 DLQSP LNPCV ILSELYVEK K YMD SKMKPL WL VYSSRAFG EDSVGVIFKN GDDL RQDMLT
841 LQMLRLMDLL WKEAGLDLRM LPYGC LATGD RSGLIEVVST SETIADIQLN SSNVAATAAF
901 NKDALLNLWK EYNSGDDLDR AIEEFTLS CA GYCVASYVLG IGD RHSDNIM VKKTGQLFHI
961 DFGHILGNFK SKFGIKRERV PFILTYDFIH VIQQGKTGNT EKFGFRFQCC EDAYLILRRH
1021 GNLFITLFAL MLTAGLPELT 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 cga aaacctg
61 tattttcagg gcgccatgga tccggaattc atgcctcctg ctatggcaga caaccttgac
121 atctgggcag tggactcaca gattgcatcc gatggcgcca tatccgtcga tttccttctg
181 cccaccggga tttatatcca gttggaagta cctcgggag ctaccatttc ttatatataa
241 cagatgttat ggaagcaagt tcacaactac ccgatgttta acctcctcat ggacattgac
301 tcgtatatgt ttgcatgtgt gaatcaaact gctgtatatg aggaactgga agacgaaaca
361 cgaagacttt gtgatgtcag accttttctt ccagttctca aactagtac tagaagctgt
421 gaccccgag aaaaattgga ctcaaaaatt ggggttctta taggaaaagg tcttcatgag
481 tttgatgcct tgaaggatcc cgaagtgaat gaatttagaa gaaaaatgcg caaattcagt
541 gagccaaga ttcagtctct ggtagggttg tcttgatcg actggctaaa gcacacgtat
601 ccgctgagc acgagccgtc cgtcctggag aacttggaag ataaacttta tggaggaaag
661 ctggttgagg ctgtgcactt tgaaaatagc caggatgtat ttagttttca agtgctctcc
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901 accctgcccc acttcatcct tgtggaatgt tgtaagatca agaaaatgta tgaacaagaa
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p85β Sequence Information

<u>Protein</u>	Murine p85β
<u>Tags</u>	Untagged
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of murine p85β
<u>Accession number</u>	GenBank BC006796

Recombinant p85β amino acid sequence:

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1  MAGAEGFQYR  AVYPFRRERP  EDLELLPGDL  LVVSRVALQA  LGVADGGERC  PHNVGWMPGF
61  NERTRQRGDF  PGTYVEFLGP  VALARPGPRP  RGPRPLPARP  LDGSSESGHI  LPDLAEQFSP
121 PDPAPPILVK  LVEAIEQAEL  DSECYSKPEL  PATRTDWSLS  DLEQWDRTAL  YDAVKGFLLA
181 LPAAVVTPEA  AAEAYRALRE  VAGPVGLVLE  PPTLPLHQAL  TLRFLQLHLG  RVARRAPSPD
241 TAVHALASAF  GPLLLRIPPS  GGECDGSEPV  PDFPVLLLER  LVQEHVEEQD  AAPPALPPKP
301 SKAKPAPTAL  ANGGSPPSLQ  DAEWYWGDIS  REEVNERLRD  TPDGTFLVRD  ASSKIQGEYT
361 LTLRKGGNNK  LIKVFHRDGH  YGFSEPLTFC  SVVELISHYR  HESLAQYNAK  LDTRLLYPVS
421 KYQQDQVVKE  DSVEAVGAQL  KVYHQYQDK  SREYDQLYEE  YTRTSQELQM  KRTAIEAFNE
481 TIKIFEEQGQ  TQEKCSKEYL  ERFRREGNEK  EMQRILLNSE  RLKSRIAEIH  ESRTKLEQDL
541 RAQASDNREI  DKRMNSLKP  LMQLRKIRDQ  YLVWLTQKGA  RQRKINELWG  IKNETEDQYS
601 LMEDEDALPH  HEERTWYVGK  INRTQAEEML  SGKRDGTFLI  RESSQRGCYA  CSVVVDGDTK
661 HCVIYRTATG  FGFAEPYNLY  GSLKELVLHY  QHASLVQHND  ALTVTLAHPV  RAPGPGPPSA
721  AR

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Recombinant p85β nucleotide sequence:

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1  atggcaggag  ccgagggcct  ccagtacagg  gctgtgtacc  cattccgccg  ggagcggcct
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1741 cgacagagga agatcaacga atggctggga atcaagaacg agactgagga ccagtattca
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2161 gcacgctaa
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Reviewed and approved by site quality representative.

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