

Certificate of Analysis

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

Item # 14-785, 14-785-K, 14-785M

Parent Lot # D8HN028U

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, full-length mouse p110 α and untagged, recombinant, full-length mouse p85 α . Co-expressed by baculovirus in Sf21 insect cells and purified using Ni²⁺/NTA-agarose.

Purity (p110 α & p85 α combined) 82.5% by SDS-PAGE and Coomassie blue staining. p110 α MW = 129kDa, p85 α MW = 83.6kDa.

Specific Activity (Parent lot# D8HN028U): 186U/mg, where one unit of PI 3-Kinase alpha (p110 α /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: 1.777mg/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.

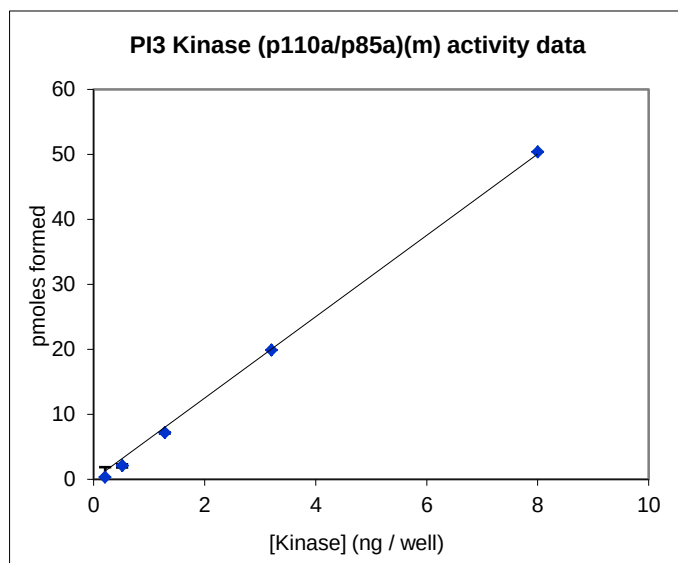
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.2–8ng 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 α /p85 α) with the p110 α and p85 α 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 PI 3-Kinase (p110 α /p85 α)



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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 α Sequence Information

Protein	Murine p110 α
Tags	N-terminal 6His
Native sequence	M37 of the recombinant sequence is equivalent to M1 of murine p110 α
Accession number	GenBank BC089038

Recombinant p110 α amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELFEAR KYPLHQLLQD ETSYIFVSVT QEAEREFFFD
121 ETRRLCDLRL FQPFLKVIEP VGNREEKILN REIGFVIGMP VCEFDMVKDP EVQDFRRNIL
181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQIIIVI WVIVSPNNDK
241 QKYTLKINH D CVPEQVIAEA IRKKTRSMML SSEQLKLCVL EYQGYILKV CGCDEYFLEK
301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
361 KSLWVINSAL RIKILCATYV NVNIRDIDKI YVRTGIYHGG EPLCDNVNTQ RVPCSNPRWN
421 EWLNYDIYIP DLPRAARLCL SICSVKGRKG AKEEHCPLAW GNINLFDYTD TLVSGKMALN
481 LWPVPHGLED LLNPIGVGTGS NPNKETPCLE LEFDWFSSV KFPDMSVIEE HANWSVSREA
541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSEIT EQEKDFLWSH RHYCVTIPEI
601 LPKLLLSVKW NSRDEVAQMY CLVKDWPPIK PEQAMELLDC NYPDPMVRSF AVRACLEKYLT
661 DDKLSQYLIQ LVQVLKYEQY LDNLLVRFL KALTNQRIG HFFFVHLKSE MHNKTVSQRF
721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEK DETQKVQMKF LVEQMRQPDF
781 MDALQGFLSP LNPALHQLGNL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIIRIMENIW QNQGLDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKGALQFHS HTLHQWLKDK NKGEIYDAAI DLFTSRSCAGY CVATFILGIG DRHNSNIMVK
961 DDGQLFHIDF GHFLDHKKKK FGYKRERVPF VLTQDFLIVI SKGAQEYTKT REFERFQEMC
1021 YKAYLAIRQH ANLFINLFSM MLGSGMPELQ SFDDIAYIRK TLALDKTEQE ALEYFTKQMN
1081 DAHHGGWTTK MDWIFHTIKQ HALN

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Recombinant p110 α nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gcctccacga
121 ccatcttcgg gtgaactgtg gggcatccac ttgatgcccc cacgaatcct agtggaatgt
181 ttactcccca atggaatgat agtgacttta gaatgcctcc gtgaggccac actcgtcacc
241 atcaaactat aactgttcag agaggccagg aaataccctc tccatcagct tctgcaagac
301 gaaacttctt acattttcgt aagtgtcacc caagaagcag aaaggaaga attttttgat
361 gaaacaagac gactttgtga ccttcggctt tttcaaccct ttttaaaagt tattgaacca
421 gtaggcaacc gtgaagaaa gatcctcaat cgagaaattg gttttgttat tggcatgcca
481 gtgtgtgaat ttgatatggt taaagatcca gaagtccaag actttcgaag gaacattctg
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601 tatgtctacc ctccaaatgt cgagctcttc ccagaactgc caaagcacat ctacaacaag
661 ttagataaag gacaaatcat agtgggtgatt tgggtaatat tctctccaaa caacgacaag
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1021 ccgtcactat ccaggcgcat ctccacagcc acaccctaca tgaatggaga gacatctacg
1081 aatccctctt gggtcataaa tagtgcgctc agaataaaaa ttctttgtgc aacctatgta
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3301 catgctttga actaa

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p85α Sequence Information

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

Recombinant p85α amino acid sequence:

1	MSAEGYQYRA	LYDYKKEREE	DIDLHLGDIL	TVNKGSLVAL	GFSDGQEARP	EDIGWLNGYN
61	ETTGERGDFP	GTIVEYIGRK	RISPTPKPR	PPRPLPVAPG	SSKTEADTEQ	QALPLPDIAE
121	QFAPPDVAPP	LLIKLLEAIE	KKGLECSTLY	RTQSSSNPAE	LRQLLDCAAA	SVDLEMIDVH
181	VLADAFKRYL	ADLPNPVIVP	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	QERYKEYIE	KFKREGNEKE	IQRIMHNHDK	LKSRISEIID
541	SRRRLEEDLK	KQAAEYREID	KRMNSIKPDL	IQLRKTRDQY	LMWLTQKQVR	QKKLNEWLGN
601	ENTEDQYSLV	EDDEDLPHHD	EKTWNVGSN	RNKAENLLRG	KRDGTFLVRE	SSKQGCYACS
661	VVVDGEVHKC	VINKTATGYG	FAEPYNLYSS	LKELVLHYQH	TSLVQHNSDL	NVTLAYPVYA
721	QQR					

Recombinant p85α nucleotide sequence:

1	atgagtgcctg	aggggtacca	gtacagagca	ctgtacgact	acaagaagga	gcgagaggaa
61	gacattgacc	tacacctggg	ggacatactg	actgtgaata	aaggctcctt	agtggcactt
121	ggattcagtg	atggccagga	agcccgccct	gaagatattg	gctgggttaa	tggctacaat
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961	agcatgaaca	acaatatgtc	cttgaggat	gctgaatggg	actggggaga	catctcaagg
1021	gaagaagtga	atgaaaaact	ccgagacact	gctgatggga	cctttttggg	acgagacgca
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1141	atcaaaatct	ttcaccgtga	tggaaaatat	ggcttctctg	atccattaac	cttcaactct
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1381	cggaatatg	atagattata	tgaggagtac	acccgtactt	cccaggaaat	ccaaatgaaa
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1501	caggagcggg	acagcaaaga	atacatagag	aagtttaaac	gcgaaggcaa	cgagaaagaa
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1801 gaaaataccg aagatcaata ctccctggta gaagatgatg aggatttgcc ccaccatgac
1861 gagaagacgt ggaatgtcgg aagcagcaac cgaaacaaag cggagaacct attgcgaggg
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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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