

Certificate of Analysis

PI 3-Kinase (p110 α (E542K)/p85 α) murine

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-791, 14-791-K, 14-791M

Parent Lot # 1606095

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 murine p110 α full length, containing the mutation E542K, and untagged, recombinant, murine p85 α full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

The E542K substitution is a somatic mutation in p110 α that has been associated with tumours of the colon and brain. Combined *in vitro* and *in vivo* studies have shown that this mutation confers higher lipid kinase activity than wild type, and is able to induce oncogenic transformation. (Kang S. *et al.*, PNAS, (2005);**102**: 802-807 and Zhao J.J. *et al.*, PNAS, (2005);**102**:18443-18448).

Purity (p110 α (E542K) and p85 α combined) 75.2% by SDS-PAGE and Coomassie blue staining. p110 α (E542K) MW = 129kDa, p85 α MW = 84kDa.

Specific Activity (Parent lot# 1606095): 436U/mg, where one unit of PI 3-Kinase (p110 α (E542K)/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: 0.728mg/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.

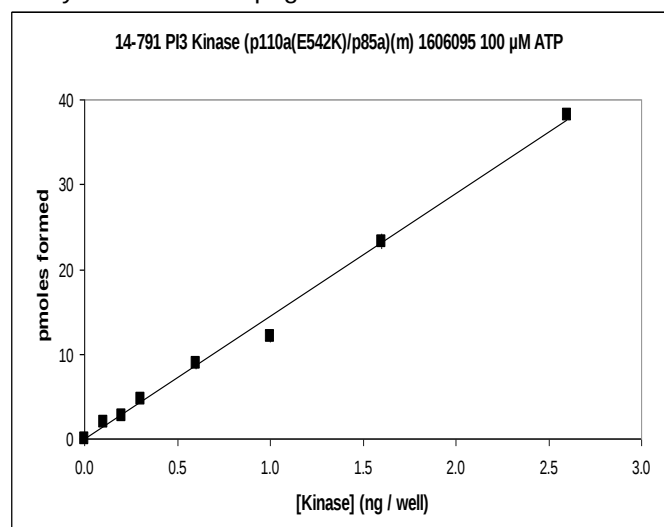
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

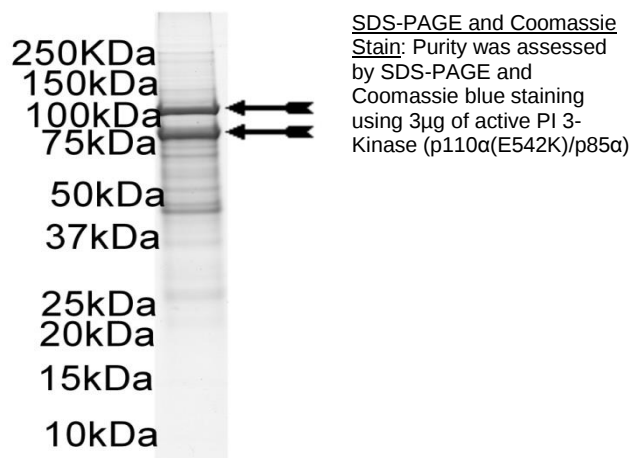
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.2–6ng 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 four and six.



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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 (E542K) Sequence Information

Protein	Murine p110 α (E542K)
Tags	N-terminal 6His
Native sequence	M37 of the recombinant protein is equivalent to M1 of murine p110 α
Accession number	GenBank BC089038

Recombinant p110 α (E542K) amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELFREAR KYPLHQLLQD ETSYIFVSVT QEAEREFFD
121 ETRRLCDLRL FQPFLKVIEP VGNREEKILN REIGFVIGMP VCEFDMVKDP EVQDFRRNIL
181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQIIVVI WVIVSPNNDK
241 QKYTLKINH D CVPEQVIAEA IRKKTSMML SSEQLKLCVL EYQGYILKV CGCDEYFLEK
301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
361 KSLWVINSAL RIKILCATYV NVNIRDIDKI YVRTGIYHGG EPLCDNVNTQ RVPCSNPRWN
421 EWLNYDIYIP DLPRAARLCL SICSVKGRKG AKEEHCLAW GNINLFDYTD TLVSGKMALN
481 LWPVPHGLED LLNPIGVGTGS NPNKETPCLE LEFDWFSSVV KFPDMSVIEE HANWSVSREA
541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSKIT EQEKDFLWSH RHYCVTIPEI
601 LPKLLLSVKW NSRDEVAQMY CLVKDWPPIK PEQAMELLDC NYPDPMVRSF AVRACLEKYL
661 DDKLSQYLIQ LVQVLKYEYQ LDNLLVRFLL KKALTNQRIG HFFFVHLKSE MHNKTVSQRF
721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEKK DETQKVQMKF LVEQMRQPDF
781 MDALQGFLSP LNPALHQLGNL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIIRIMENIW QNQGLDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKGALQFNS HTLHQWLKDK NKGEIYDAAI DLFTRSCAGY 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 (E542K) nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcgcattga tccggaattc aaaggcctac gtcgacaaat gcctccacga
121 ccattctcgg gtgaactgtg gggcatccac ttgatgcccc cacgaatcct agtgggaatgt
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301 gaaacttctt acattttcgt aagtggtcacc caagaagcag aaagggaaga attttttgat
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421 gtaggcaacc gtgaagaaaa gatcctcaat cgagaaattg gttttgttat tggcatgcca
481 gtgtgtgaat ttgatatggt taaagatcca gaagtccaag actttcgaag gaacattctg
541 aatgtttgca aagaagctgt ggacctgcgg gatctcaact cgcctcatag cagagcaatg
601 tatgtctacc ctccaaatgt cgagtcttcc ccagaactgc caaagcacat ctacaacaag
661 ttagataaag gacaaatcat agtgggtgatt tgggtaatag tctctccaaa caacgacaag
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781 atcaggaaaa agactcggag catgttgttg tcctctgagc agctgaaact ctgtgtctta
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1381 ggaacataaa acttgtttga ttatacagac accctagtgt ccgggaaaat ggctttgaat
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3181 actctagcct tggacaaaac tgagcaagaa gctttggaat atttcacaaa gcaaatgaat
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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:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EDIGWLNGYN
61 ETTGERGDFP GTYVEYIGRK RISPTPKPR PPRPLPVAPG SSKTEADTEQ QALPLPDIAE
121 QFAPPDVAPP LLIKLLEAIE KKGLECSLY RTQSSSNPAE LRQLDCDAA SVDLEMIDVH
181 VLADAFKRYL ADLPNPVIPV AVYNEMMSLA QELQSPEDCI QLLKKLIRLP NIPHCWLT
241 QYLLKHFFKL SQASSKNLLN ARVLSEIFSP VLFRFPAASS DNTEHLIKAI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN SMNNNMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDDLTFNS VVELINHYRN ESQAQYNPKL
421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEK REYDRLYEEY TRTSQEIQMK
481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
601 ENTEDQYSLV EDDELPHHD EKTWNVGSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
661 VVDGEVKHC VINKTATGYG FAEPNLYSS LKELVLHYQH TSLVQHNSL 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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1621 agtaggagga ggttgaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac
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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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