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Certificate of Analysis

PI 3-Kinase (p110α(E545K)/p85α), murine

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-781, 14-781-K, 14-781M
Parent Lot # D7NN042U

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 p110α full length, containing the mutation E545K, and untagged, recombinant, murine p85α full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose.
The E545K substitution is a somatic mutation in p110α 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 (p110α and p85α combined) 75% by SDS-PAGE and Coomassie blue staining. p110α MW = 129kDa, p85α MW = 83.6kDa.

Specific Activity (Parent lot# D7NN042U): 665U/mg, where one unit of PI 3-Kinase (p110α(E545K)/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: 1.54mg/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.

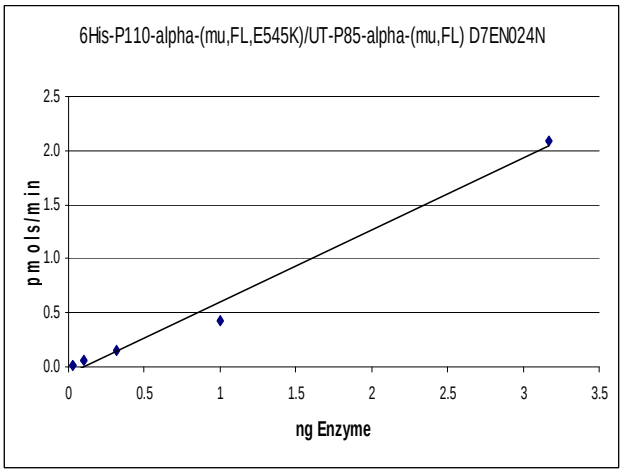
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

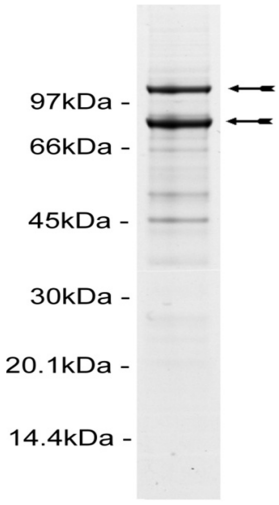
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.1–3.0ng of this enzyme phosphorylated 10µM phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.



MS Tryptic Fingerprint: Confirmed identity as PI 3-Kinase (p110α/p85α) with the p110α and p85α translated sequences listed on pages three and five.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PI 3-Kinase (p110α(E545K)/p85α), active

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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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p110α(E545K) Sequence Information

Protein Murine p110α(E545K)

Tags *N*-terminal 6His

Native sequence M37 of the recombinant protein is equivalent to M1 of murine p110α

Accession number GenBank BC089038

Recombinant p110α(E545K)amino acid sequence:

```
   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
  61 LLPNGMIVTL ECLREATLVT IKHELFREAR KYPLHQLLQD ETSYIFVSVT QEAEREEFFD
 121 ETRRLCDLRL FQPFLKVIEP VGNREEKILN REIGFVIGMP VCEFDMVKDP EVQDFRRNIL
 181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQIIVVI WVIVSPNNDK
 241 QKYTLKINHD CVPEQVIAEA IRKKTRSMLL SSEQLKLCVL EYQGKYILKV CGCDEYFLEK
 301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
 361 KSLWVINSAL RIKILCATYV NVNIRDIDKI YVRTGIYHGG EPLCDNVNTQ RVPCSNPRWN
 421 EWLNYDIYIP DLPRAARLCL SICSVKGRKG AKEEHCPLAW GNINLFDYTD TLVSGKMALN
 481 LWPVPHGLED LLNPIGVTGS NPNKETPCLE LEFDWFSSVV KFPDMSVIEE HANWSVSREA
 541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSEIT KQEKDFLWSH RHYCVTIPEI
 601 LPKLLLSVKW NSRDEVAQMY CLVKDWPPIK PEQAMELLDC NYPDPMVRSF AVRCLEKYLT
 661 DDKLSQYLIQ LVQVLKYEQY LDNLLVRFLL KKALTNQRIG HFFFWHLKSE MHNKTVSQRF
 721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEKK DETQKVQMKF LVEQMRQPDF
 781 MDALQGFLSP LNPAHQLGNL 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 p110α(E545K)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 atcaaacatg aactgttcag agaggccagg aaataccctc tccatcagct tctgcaagac
 301 gaaacttctt acattttcgt aagtgtcacc caagaagcag aaagggaaga atttttgat
 361 gaaacaagac gactttgtga ccttcggctt tttcaaccct ttttaaaagt tattgaacca
 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 agtggtgatt tgggtaatag tctctccaaa caacgacaag
 721 cagaagtaca ctctgaagat caatcatgac tgtgtgccag agcaagtcat tgctgaagcc
 781 atcaggaaaa agactcggag catgttgttg tcctctgagc agctgaaact ctgtgtctta
 841 gaatatcagg gcaagtatat tctgaaagtg tgtggctgtg acgaatactt cctggaaaag
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 961 ttgatgctga tggccaaaga aagcctatac tctcagctgc cgattgatag cttcaccatg
1021 ccgtcatact ccaggcgcat ctccacagcc acaccctaca tgaatggaga gacatctacg
1081 aaatccctct gggtcataaa tagtgcgctc agaataaaaa ttctttgtgc aacctatgta
1141 aatgtaaata ttcgagacat tgataagatc tatgttcgaa caggtatcta ccatggagga
1201 gaacccttat gtgacaatgt gaacactcaa agagtacctt gttccaatcc taggtggaat
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1381 ggaaacataa acttgtttga ttatacagac accctagtgt ccgggaaaat ggctttgaat
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1561 aagtttccag acatgtctgt gatcgaagaa catgccaatt ggtccgtgtc ccgagaagct
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1681 gaaaatgaca aggaacagct ccgagcactt tgcacccggg acccactatc tgaaatcact
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1861 tgcttagtaa aagattggcc tccaatcaaa ccagagcaag ccatggaact cctggactgt
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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

Protein Murine p85α

Tags Untagged

Native sequence M1 of the recombinant protein is equivalent to M1 of murine p85α

Accession number GenBank NM_001077495

Recombinant p85α amino acid sequence:

```
   1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSDGQEARP EDIGWLNGYN
  61 ETTGERGDFP GTYVEYIGRK RISPPTPKPR 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 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDPLTFNS VVELINHYRN ESLAQYNPKL
 421 DVKLLYPVSK YQQDQVVKED NIEAVGKKLH EYNTQFQEKS REYDRLYEEY TRTSQEIQMK
 481 RTAIEAFNET IKIFEEQCQT QERYSKEYIE KFKREGNEKE IQRIMHNHDK LKSRISEIID
 541 SRRRLEEDLK KQAAEYREID KRMNSIKPDL IQLRKTRDQY LMWLTQKGVR QKKLNEWLGN
 601 ENTEDQYSLV EDDEDLPHHD EKTWNVGSSN RNKAENLLRG KRDGTFLVRE SSKQGCYACS
 661 VVVDGEVKHC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNDSL NVTLAYPVYA
 721 QQRR
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Recombinant p85α nucleotide sequence:

```
   1 atgagtgctg aggggtacca gtacagagca ctgtacgact acaagaagga gcgagaggaa
  61 gacattgacc tacacctggg ggacatactg actgtgaata aaggctcctt agtggcactt
 121 ggattcagtg atggccagga agcccggcct gaagatattg gctggttaaa tggctacaat
 181 gaaaccactg gggagagggg agactttcca ggaacttacg ttgaatacat tggaaggaaa
 241 agaatttcac cccctactcc caagcctcgg ccccctcgac cgcttcctgt tgctccgggt
 301 tcttcaaaaa ctgaagctga cacggagcag caagcgttgc cccttcctga cctggccgag
 361 cagtttgccc ctcctgatgt tgccccgcct ctccttataa agctcctgga agccattgag
 421 aagaaaggac tggaatgttc gactctatac agaacacaaa gctccagcaa ccctgcagaa
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 541 gtcttagcag atgctttcaa acgctatctc gccgacttac caaatcctgt cattcctgta
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1621 agtaggagga ggttggaaga agacttgaag aagcaggcag ctgagtaccg agagatcgac
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1741 ttgatgtggc tgacgcagaa aggtgtgcgg cagaagaagc tgaacgagtg gctggggaat
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2101 acctccctcg tgcagcacaa tgactccctc aatgtcacac tagcataccc agtatatgca
2161 caacagaggc gataa
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Reviewed and approved by site quality representative.