

Certificate of Analysis

PI 3-Kinase (p110 α (E545K)/p85 α), murine
(recombinant enzyme expressed in Sf21 insect cells)

Catalog # 14-781M

Lot # D7NN042U

Product Description: Complex of *N*-terminal 6His-tagged recombinant murine p110 α , full length, containing the mutation E545K, and untagged, recombinant, murine p85 α , full length. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

The E545K 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 and p85 α combined) 75% by SDS-PAGE and Coomassie blue staining. p110 α MW = 129 kDa, p85 α MW = 83.6 kDa.

Specific Activity (Lot # D7NN042U): 656 U/mg, where one unit of PI 3-Kinase (p110 α (E545K)/p85 α) activity is defined as 1 nmol phosphatidylinositol 3,4,5-trisphosphate (PIP3) formed per minute at room temperature with a final ATP concentration of 100 μ M. Calculated U/mg was determined after conversion of the HTRF ratio to pmol formed using the PIP3 calibration curve described below.

Formulation: 250 μ g of enzyme in 162.7 μ l of 50 mM Tris/HCl pH7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 0.2 mM PMSF, 1 mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

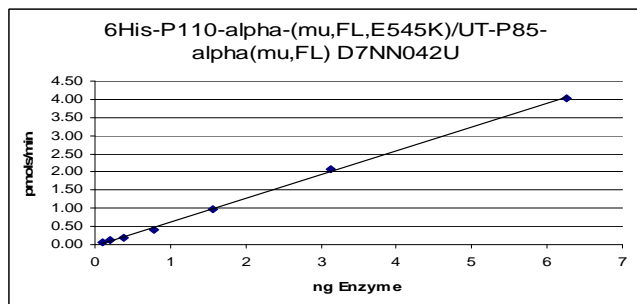
Storage and Stability: Store at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

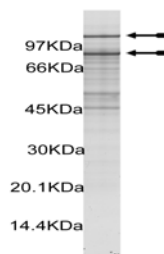
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.1 - 6 ng of this lot of PI 3-kinase alpha (p110 α (E545K)/p85 α) was assayed in a 30 minute reaction using the PIPProfiler™ protocol described on page 2



MS Tryptic Fingerprint: Confirmed identity as PI 3-Kinase (p110 α /p85 α) with 26% p110 α and 26% p85 α amino acid coverage of the translated sequences listed on pages 3 and 5.



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 (E545K)/p85 α)

Kinase Assay Protocol

Protocol for the PI 3-K HTRF™ assay (catalog # 33-017) found at <http://www.millipore.com/coa/tech1/74jt4z>, summarized below

1. Add 5 µl of 60 µM phosphatidylinositol 4,5-bisphosphate (PIP2) substrate.
2. Add 10 µl (0.1– 6ng) PI 3-Kinase (p110α(E545K)/p85α).
3. Add 5 µl 400 µM ATP.
4. Incubate for 30 minutes at room temperature.
5. Add 5 µl Stop Buffer containing biotinylated-PIP3 and EDTA.
6. Add 5 µl Detection Buffer containing Europium labeled anti-GST monoclonal antibody, GST tagged GRP1 PH domain, and Streptavidin-Allophycocyanin.
7. Read plate in HTRF mode. Product formed should be determined from a standard curve of non-biotinylated PIP3.

Generation of PIP3 calibration curve

This optional calibration should be performed on the same plate as the enzyme assay to enable appropriate conversion to pmol formed where desired.

1. Dilute PIP3 in 1 x reaction buffer containing PIP2 substrate. Start concentration curve from 10 µM (final assay concentration) and dilute in semi-log steps for 12 points. Include a zero PIP3 point.
2. Add 15 µl of each dilution point to appropriate wells of a black 384-well microtitre plate.
3. Add 5 µl of ATP to all wells.
4. Add 5 µl Stop Buffer containing biotinylated-PIP3 and EDTA.
5. Add 5 µl Detection Buffer containing Europium labeled anti-GST monoclonal antibody, GST tagged GRP1 PH domain, and Streptavidin-Allophycocyanin.
6. Read plate in HTRF mode and fit data to a four-parameter logistic.

p110 α (E545K) Sequence Information

Protein Murine p110 α (E545K)
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 (E545K) amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMPPR PSSGELWGIH LMPPRILVEC
61 LLPNGMIVTL ECLREATLVT IKHELRFREAR KYPLHQLLQD ETSYIFVSVT QEAEEREEFFD
121 ETRRLCDLRL FQPFLKVIIEP VGNREEKILN REIGFVIGMP VCEFDMVKDP EVQDFRRNIL
181 NVCKEAVDLR DLNSPHSRAM YVYPPNVESS PELPKHIYNK LDKGQIIVVI WVIIVSPNNDK
241 QKYTLKINHND CVPEQVIAEA IRKKTRSMML SSEQLKLCVL EYQGYILKV CGCDEYFLEK
301 YPLSQYKYIR SCIMLGRMPN LMLMAKESLY SQLPIDSFTM PSYSRRISTA TPYMNGETST
361 KSLWVINSAL RIKILCATYV NVNIRDIDKI YVRTGIYHGG EPLCDNVNTQ RVPASNPRWN
421 EWLNYDIYIP DLPRAARLCL SICSVKGRKG AKEEHCPLAW GNINLFDYTD TLVSGKMALN
481 LWPVPHGLED LLNPIGVGTGS NPNKETPCLE LEFDWFSSVV KFPDMSVIEE HANWSVSREA
541 GFSYSHTGLS NRLARDNELR ENDKEQLRAL CTRDPLSEIT KQEKDFLWSH RHYCVTIPEI
601 LPKLLLSVKW NSRDEVAQMY CLVKDWPPIK PEQAMELLDC NYPDPMVRSF AVRCLKEYLT
661 DDKLSQYLIQ LVQVLKYEQY LDNLLVRFLL KKALTNQRIG HFFFVHLKSE MHNKTVSQRF
721 GLLLESYCRA CGMYLKHLNR QVEAMEKLIN LTDILKQEKK DETQKVMQKF LVEQMRQPDF
781 MDALQGFLSP LNPAAQLGNL RLEECRIMSS AKRPLWLNWE NPDIMSELLF QNNEIIFKNG
841 DDLRQDMLTL QIRIMENIW QNQLDLRML PYGCLSIGDC VGLIEVVRNS HTIMQIQCKG
901 GLKGALQFNS HTLHQWLKDK NKGEIYDAI 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 (E545K) nucleotide sequence:

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1 atgtcgact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
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3301 catgctttga actaa

p85 α Sequence Information

Protein Murine p85 α
Tags Untagged
Native sequence M1 of the recombinant protein is equivalent to M1 of murine p85 α
Accession number GenBank NM_001077495

Recombinant p85 α amino acid sequence:

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1 MSAEGYQYRA LYDYKKEREE DIDLHLGDIL TVNKGSLVAL GFSGDQEARP EDIGWLNGYN
61 ETGGERGDFP GTYVEYIGRK RISPPTPKPR PPRPLPVAPG SSKTEADTEQ QALPLPDLAE
121 QFAPPDVAPP LLIKLLLEAIE KKGLECSTLY RTQSSSNPAE LRQLLDCDAA SVDLEMIDVH
181 VLADAFKRYL ADLPNPVIPV AVYNEMMSLA QELQSPEDCI QLLKKLIRLP NIPHQCWLTL
241 QYLLKHFFKL SQASSKNLLN ARVLSEIFSP VLFRFPAASS DNTEHLIKAI EILISTEWNE
301 RQPAPALPPK PPKPTTVANN SMNNSMSLQD AEWYWGDISR EEVNEKLRDT ADGTFLVRDA
361 STKMHGDYTL TLRKGGNNKL IKIFHRDGKY GFSDDLTFNS VVELINHYRN ESLAQYNPKL
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601 ENTEDQYSLV EDDLDLPHHD EKTWNVGS SNRNKAENLLRG KRDTGTFVRE SSKQGCYACS
661 VVVDGEVHKC VINKTATGYG FAEPYNLYSS LKELVLHYQH TSLVQHNSL NVTLAYPVYA
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Recombinant p85 α nucleotide sequence:

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1 atgagtgctg aggggtacca gtacagagca ctgtacgact acaagaagga gcgagaggaa
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