

Certificate of Analysis

PIK3C3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-940, 14-940-K, 14-940M

Parent Lot # D13EP003N

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: N-terminal 6His-tagged, recombinant, human PIK3C3 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 90% by SDS-PAGE and Coomassie blue staining. MW = 105kDa.

Specific Activity (Parent lot# D13EP003N): 242U/mg, where one unit of PIK3C3, active activity is defined as 1nmol phosphatidylinositol 3-phosphate (PI(3)P) formed per minute at room temperature with a final ATP concentration of 200µM.

Formulation: 2.110mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 20% glycerol. Frozen solution.

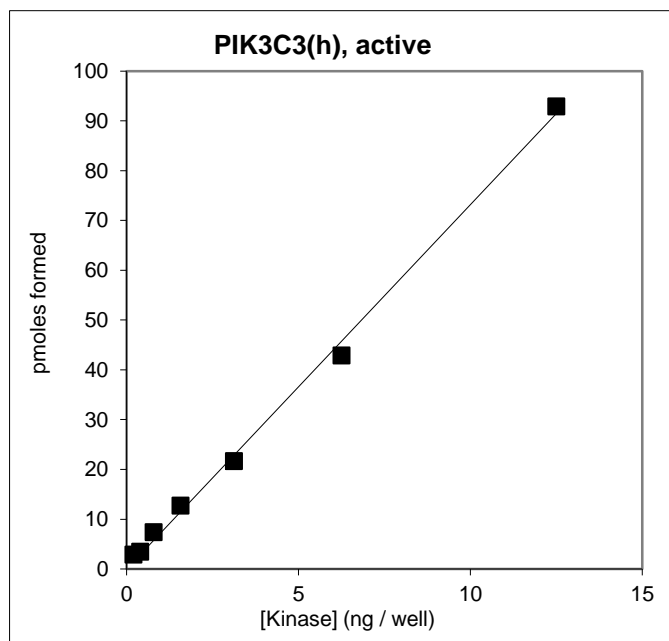
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

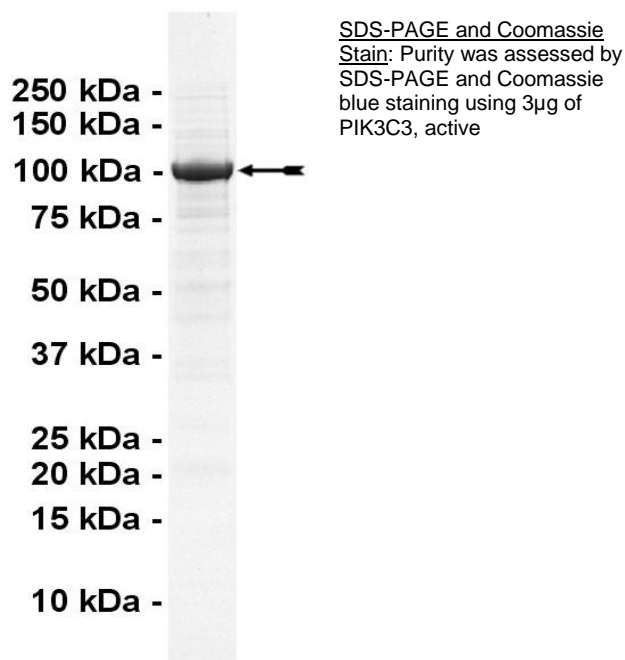
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.2–12.5ng of this lot of enzyme phosphorylated 25µM phosphatidylinositol in the assay referenced on page two.



MS Tryptic Fingerprint: Confirmed identity as PIK3C3 with the translated sequence listed on page three.



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Eurofins PI3 Kinase Homogeneous Time-resolved Fluorescence (HTRF) Class II Reagent Kits

The following Eurofins kits are suitable for use with this enzyme. Assay protocol can be found in the corresponding Certificate of Analysis.

Cat. No	Kit Description
33-038	PI 3-Kinase (Class II) HTRF Assay 1-plate kit
33-039	PI 3-Kinase (Class II) HTRF Assay 5-plate kit

14-940-K PIK3C3(h), active is suitable for use with the Class II HTRF assay kit except that it requires an alternative Reaction Buffer. The final concentration of the alternative Reaction Buffer is 50 mM Hepes, pH 7.0, 0.0125% Sodium Azide, 3 mM MnCl₂ and 0.3 mg/ml BSA and this can be substituted for the 1x Reaction Buffer in the Class II kit.

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PIK3C3 Sequence Information

<u>Protein</u>	human PIK3C3
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human PIK3C3
<u>Accession number</u>	GenBank BC053651

Recombinant PIK3C3 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MGEAEKFHYI YSCDLINQVQ LKIGSLEGKR
61 EQKSYKAVLE DPMLKFSGLY QETCSPLYVT CQVFAEGKPL ALPVRTSYKA FSTRWNWNEW
121 LKLPVKYPLD PRNAQVALTI WDVYGPVKAV PVGGTTVSLF GKYGMFRQGM HDLKVWPNVE
181 ADGSEPTKTP GRTSSTLSED QMSRLAKLTK AHRQGHMVKV DWLDRLTFRE IEMINESEKR
241 SSNFMYLMVE FRCVKCDDKE YGIVVYEKDG DESSPILTSF ELVKVPDPQM SMENLVESKH
301 HKLARSLRSG PSDHDLKPNA ATRDQLNIIV SYPPTKQLTY EEQDLVWVFR YYLTNQEKAL
361 TKFLKCVNWD LPQEAQALE LLGKWKPM DV EDSLELLSSH YTNPTVRRYA VARLRQADDE
421 DLLMYLLQLV QALKYENFDD IKNGLEPTTK DSQSSVSENV SNSGINS AEI DSSQIITSPL
481 PSVSSPPPAS KTKEVPDGEN LEQDLCTFLI SRACKNSTLA NYLYWYVIVE CEDQDTQQRD
541 PKTHEMYLNV MRRFSQALLK GDKSVRVMRS LLAAQQT FVD RLVHLMKAVQ RESG NRKKKN
601 ERLQALLGDN EKMNLSDEL IPLPLEPVK IRGIIPETAT LFKSALMPAQ LFFKTEDGGK
661 YPVIFKHGDD LRQDQLILQI ISLMDKLLRK ENLDLKLTPY KVLATSTKHG FMQFIQSVPV
721 AEVLDTESI QNFFRKYAPS ENGPNGISAE VMDTYVKSCA GYCVITYILG VGDRHLDNLL
781 LTKTKGLFHI DFGYILGRDP KPLPPMKNL KEMVEG MGT QSEQYQEFRK QCYTAFHLHR
841 RYSNLILNLF SLMVDANIPD IALEPDKTVK KVQDKFRLDL SDEEAVHYMQ SLIDESVHAL
901 FAAVVEQIHK FAQYWRK
    
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Recombinant PIK3C3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tccaacgac cgaaaacctg
61 tatttttcagg gcgcatgga tccggaattc atgggggaag cagagaagtt tcaactacatc
121 tatagttgtg acctggatat caacgtccag cttagatag gaagcttga agggaaagaga
181 gaacaaaaga gttataaagc tgcctggaa gaccaatgt tgaagtctc aggactatat
241 caagagacat gctctgatct ttatgttact tgtcaagttt ttgcagaagg gaagcctttg
301 gccttgccag tgagaacatc ctacaaagca tttagtacaa gatggaactg gaatgaatga
361 ctgaaactac cagtaaaata cctgacctg cccaggaatg cccaagtggc cctcaccata
421 tgggatgtgt atgggtcccgg aaaagcagtg cctgtaggag gaacaacggg ttcgctcttt
481 ggaaaatacg gcatgtttcg ccaagggatg catgacttga aagtctggcc taatgtagaa
541 gcagatggat cagaaccac aaaaactcct ggagaacaa gtagcactct ctcagaagat
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721 agttctaatt tcatgtacct gatggtttaa tttcgatgtg tcaagtgtga tgataaggaa
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961 gccacgagag atcagttaaa tattattgtg agttatccac caaccaagca acttacatat
1021 gaagaacaag atcttgtttg gaagtttaga tattatctta cgaatcaaga aaaagccttg
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1141 cttctgggaa aatggaagcc gatggatgta gaggactcct tggagctgtt atcctctcat
1201 tacaccaacc caactgtgag gcgttatgct gttgccggg tgcgacaggc cgatgatgag
1261 gatttgttga tgtacctatt acaattggtc caggctctca aatatgaaa ttttgatgat
1321 ataaagaatg gattggaacc taccaagaag gatagtcaga gttcagtgct agaaaatgtg
    
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1381 tcaaattctg gaataaattc tgcagaaata gatagctccc aaattataac cagccccctt
1441 ctttcagtct cttcacctcc tcctgcatca aaaacaaaag aagttccaga tggcgaaaat
1501 ctggaacaag atctctgtac cttcttgata tcgagagcct gcaaaaactc aacactggct
1561 aattatttat actggtatgt gatagtggaa tgtgaagatc aagatactca gcagagagat
1621 ccaaagacc atgagatgta cttgaacgta atgagaagat tcagccaagc attggtgaag
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1741 cggttggtgc atctaatagaa ggcagtacaa cgcgaaagtg gaaatcgtaa gaaaaagaat
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1921 ctgtttaaaa gtgcccttat gcctgcacag ttgtttttta agacggaaga tggaggcaaa
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2101 aagggtttag ccaccagtac aaaacatggc ttcatgcagt ttatccagtc agttcctgtg
2161 gctgaagttc ttgatacaga ggggaagcatt cagaactttt ttagaaaata tgcaccaagt
2221 gagaatgggc caaatgggat tagtgctgag gtcatggaca cttacgttaa aagctgtgct
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2701 tttgctgcag tgggtgaaca gattcacaag tttgccagc actggagaaa ataa
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Reviewed and approved by site quality representative.

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