

Certificate of Analysis

PI4KIII α , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-908, 14-908-K, 14-908M

Parent Lot # D9KN031N

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 FLAG-tagged, recombinant human PI4KIII α full length. Expressed by baculovirus in Sf21 insect cells. Purified using FLAGTM agarose.

Purity 65% by SDS-PAGE and Coomassie blue staining. MW = 233kDa.

Specific Activity (Parent lot# D9KN031N): 427U/mg, where one unit of PI4KIII α , active activity is defined as 1nmol phosphatidylinositol 4-phosphate (PI(4)P) formed per minute at room temperature with a final ATP concentration of 100 μ M.

Formulation: 0.375mg/ml of enzyme in 50mM Tris/HCl pH7.4, 150mM NaCl, 150 μ g/ml FLAGTM peptide, 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.

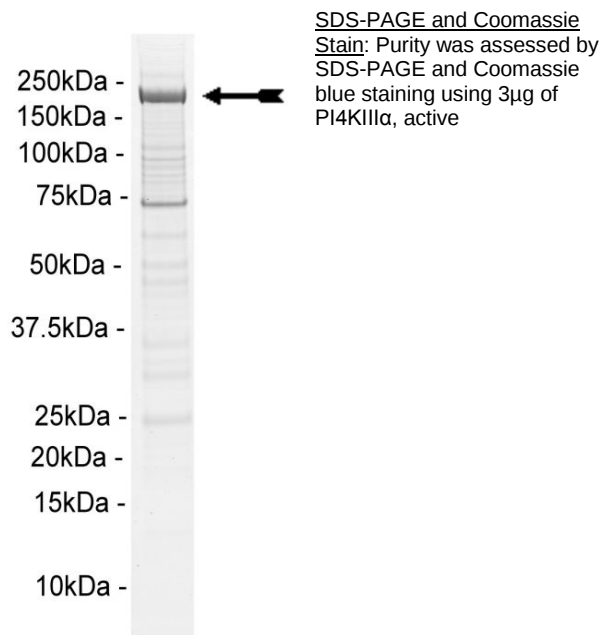
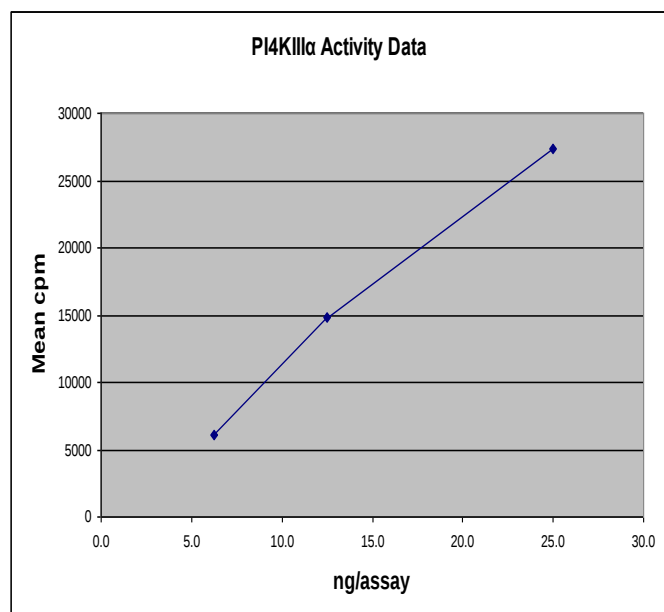
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 6–25ng of this lot of PI4KIII α was assayed in a 30 minute reaction using the protocol described on page two.

MS Tryptic Fingerprint: Confirmed product identity as PI4KIII α with the translated sequence listed on page three.



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Kinase Assay Protocol

Solutions:

1. **4 x Reaction Buffer:** Catalogue # 33-022.
2. **Substrate:** Use at a final assay concentration of 25µM phosphatidylinositol (PI) and 75µM phosphatidylserine (PS). Prepare in 4x Reaction buffer to give a 2x working stock.
3. **Wash Buffer 1:** 50mM Tris pH7.4, 80mM KCl, 10mM MgCl₂, 0.4mM EGTA.
4. **Wash Buffer 2:** 1% orthophosphoric acid, 1M NaCl.
5. **Stop Solution:** 0.5M EDTA, 1% orthophosphoric acid.
6. **35% Ethanol**
7. **PI4KIIIα, active:** Dilute with 1x Reaction buffer. Use 6–25ng per assay point.
8. **[γ-³³P]ATP:** 2.5 x MgAc/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 12.5µl of PI/PS solution to required wells.
2. Add **2.5µl (6–25ng) PI4KIIIα, active.**
3. Add 10µl of [γ-³³P]ATP.
4. Incubate for 30 minutes at RT.
5. Stop the reaction by adding 10µl of Stop solution.

Filter Plate Preparation:

6. Pre-wet all wells of an Immobilon P Multiscreen™ Plate (Catalogue # MSIPN4B10) with 15µl of 35% Ethanol.
7. Remove the ethanol by tapping plate on tissue paper.
8. Wash all wells with 200µl wash buffer 1. Remove buffer by inverting plate over a sink and tapping dry on tissue paper. Repeat this wash step twice to remove residual ethanol.

Product Capture

9. Transfer 10µl of stopped assay to appropriate wells of the prepared Immobilon P plate. Add 10µl of water to any well that does not receive a stopped reaction.
10. Using a vacuum manifold, draw liquid through membrane.
11. Wash wells 4x with 50µl of wash buffer 2, drawing liquid through the membrane by vacuum.

Counting

12. Add 40µl of an appropriate scintillation fluid to used wells and allow to stand for 30 minutes at RT.
13. Count in a scintillation counter.

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PI4KIII α Sequence Information

<u>Protein</u>	Human PI4KIII α
<u>Tags</u>	N-terminal FLAG
<u>Native sequence</u>	M17 of the recombinant protein is equivalent to M1 of PI4KIII α
<u>Accession number</u>	GenBank NM_058004

Recombinant PI4KIII α amino acid sequence:

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1  MGGDYKDDDD KARALCMCPV DFHGIFQLDE RRRDAVIALG IFLIESDLQH KDCVVPYLLR
61  LLKGLPKVYW VEESTARKGR GALPVAESFS FCLVTLLSDV AYRDP SLRDE ILEVLLQVLH
121 VLLGMCQALE IQDKEYLCKY AIPCLIGISR AFGRYSNMEE SLLSKLFPKI PPHSLRVLEE
181 LEGVRRRSFN DFRSILPSNL LTVCQEGTLK RKTSSVSSIS QVSPERGMP PSSPGGSAFH
241 YFEASCLPDG TALEPEYYFS TISSFSVSP LFNGVTYKEF NIPLEMLREL LNLVKKIVEE
301 AVLKSLDAIV ASVMEANPSA DLYYTSFSDP LYLTMFKMLR DTLYYMKDLP TSFVKEIHDF
361 VLEQFNTSQG ELQKILHDAD RIHNELSPLK LRCQANAACV DLMVWAVKDE QGAENLCIKL
421 SEKLQSKTSS KVIAHLPLL ICCLQGLGRL CERFPVVVHS VTPSLRDFLV IPSPVLVKLY
481 KYHSQYHTVA GNDIKISVTN EHSESTLNV MSGKKSQPSMY EQLRDIAIDN ICRCLKAGLT
541 VDPVIVEAFL ASLSNRLYIS QESDKDAHLI PDHTIRALGH IAAVALRDT PKVMEPIQLIQ
601 QKFCQPPSPL DVLIIDQLGC LVITGNQYIY QEVWNLFQQI SVKASSVVYS ATKDYKDHGY
661 RHCSLAVINA LANIAANIQD EHLVDELLMN LLELFVQLGL EGKRASERAS EKGPAKASS
721 SAGNLGVLIP VIAVLTRRLP PIKEAKPRLQ KLFRDFWLYS VLMGFAVEGS GLWPEEWYEG
781 VCEIATKSPL LTFPSKEPLR SVLQYNSAMK NDTVTPAELS ELRSTIINLL DPPPEVSALI
841 NKLD FAMSTY LLSVYRLEYM RVL RSTDPDR FQVMFCYFED KAIQKDKSGM MQCVIAVADK
901 VFDAFLNMMA DKAKTKENEE ELERHAQFLL VNFNHIHKRI RRVADKYL SG LVDFKPHLLW
961 SGTVLKTM LD ILQTL SLSLS ADIHKDQPYYP DIPDAPYRIT VPDTYEARES IVKDFAARCG
1021 MILQEAMKWA PTVTKSHLQE YLNKHQNWVS GLSQHTGLAM ATESILHFAG YNKQNTTLGA
1081 TQLSERPACV KKDYSNFMAS LNLNRNYAGE VYGMIRFSGT TGQMSDLNKM MVQDLHSALD
1141 RSH PQHYTQA MFKLTAMLIS SKDCDPQLLH HLCWGPLRMF NEHGMETALA CWEWLLAGKD
1201 GVEVPFMREM AGAWHMTVEQ KFG LFSAEIK EADPLAASEA SQPKPCPPEV TPHYIWIDFL
1261 VQRFEIAKYC SSDQVEIFSS LLQRSMSLNI GGAKGSMNRH VAAIGPRFKL LTLGLSLLHA
1321 DVVPNATIRN VLREKIYSTA FDYFSCPPKF PTQGEKRLRE DISIMIKFWT AMFSDKKYLT
1381 ASQLVPPDNQ DTRSNLDITV GSRQATQGW INTYPLSSGM STISKKSGMS KKTNRGSQLH
1441 KYMKRRTLL LSLATEIER LITWYNPLSA PELELDQAGE NSVANWRSKY ISLSEKQWKD
1501 NVNLAWSISP YLAVQLPARF KNTEAIGNEV TRLVRLDPGA VSDVPEAIKF LVTWHTIDAD
1561 APELSHVLCW APTDPPTGLS YFSSMYPPHP LTAQYGVKVL RSFPDAILF YIPQIVQALR
1621 YDKMGYVREY ILWAASKSQL LAHQFIWNMK TNIYLDEEGH QKDPDIGDLL DQLVEEITGS
1681 LSGPAKDFYQ REFDFFNKIT NVSAIIPYP KGDERKKACL SALSEVKVQP GCYLP SNPEA
1741 IVLDIDYKSG TPMQSAAKAP YLAKFKVKRC GVSELEKEGL RCRSDSEDEC STQEADGQKI
1801 SWQAAIFKVG DDCRQDMLAL QIIDLFKNIF QLVGLDLFVF PYRVVATAPG CGVIECIPDC
1861 TSRDQLGRQT DFGMYDYFTR QYGDESTLAF QQARYNFIRS MAAYSLLLFL LQIKDRHNGN
1921 IMLDKKGHII HIDFGFMFES SPGGNLGWEP DIKLTDEMVM IMGGKMEATP FKWFMEMCVR
1981 GYLAVRPYMD AVVSLVTLML DTGLPCFRGQ TIKLLKHRFS PNMTEREAA N FIMKVIQSCF
2041 LSNRSRTYDM IQYYQNDIPY

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Recombinant PI4KIII α nucleotide sequence:

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61  gatttccatg ggatcttcca gttagatgaa agacggagag atgcagtgat tgcattgggc
121  atttttctga ttgaatctga tcttcagcac aaagattgtg tggttcctta ctttcttcga
181  cttctcaaag gtcttccaaa agtgatttgg gtagaagaaa gcacagctcg gaaaggcaga

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6181 tga

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