

## Certificate of Analysis

### PI4KIII $\alpha$ , 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 $\alpha$  full length. Expressed by baculovirus in Sf21 insect cells. Purified using FLAG<sup>TM</sup> agarose.

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

**Specific Activity (Parent lot# D9KN031N):** 427U/mg, where one unit of PI4KIII $\alpha$ , 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 $\mu$ M.

**Formulation:** 0.375mg/ml of enzyme in 50mM Tris/HCl pH7.4, 150mM NaCl, 150 $\mu$ g/ml FLAG<sup>TM</sup> 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.

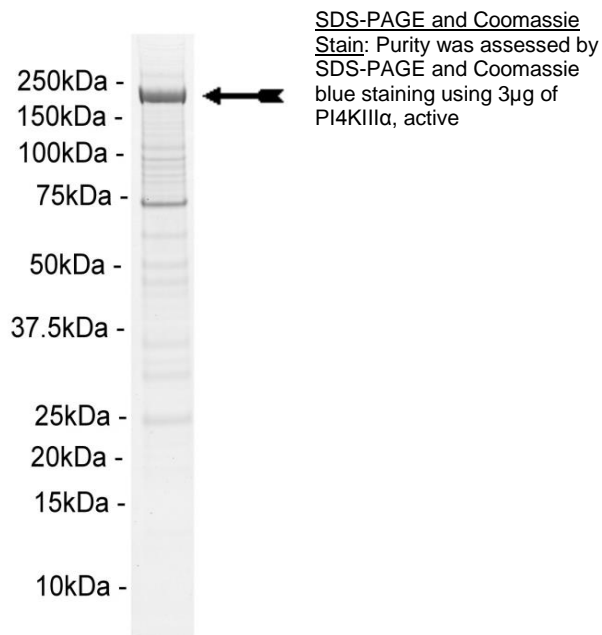
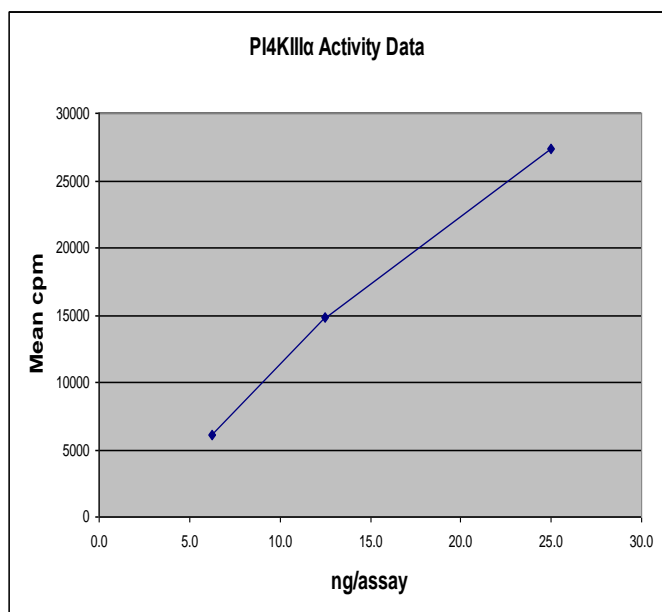
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 $\alpha$  was assayed in a 30 minute reaction using the protocol described on page two.

**MS Tryptic Fingerprint:** Confirmed product identity as PI4KIII $\alpha$  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 MgCl<sub>2</sub>, 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. **PI4KIII $\alpha$ , active:** Dilute with 1x Reaction buffer. Use 6–25ng per assay point.
8. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]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) PI4KIII $\alpha$ , active.**
3. Add 10µl of [ $\gamma$ -<sup>33</sup>P]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 $\alpha$ Sequence Information

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

#### Recombinant PI4KIII $\alpha$ amino acid sequence:

```

1  MGGDYKDDDD  KARALCMCPV  DFHGIFQLDE  RRRDAVIALG  IFLIESDLQH  KDCVVPYLLR
61  LLKGLPKVYW  VEESTARKGR  GALPVAESFS  FCLVTLSDV  AYRDP SLRDE  ILEVLLQVLH
121 VLLGMCQALE  IQDKEYLCKY  AIPCLIGISR  AFGRYSNMEE  SLLSKLFPKI  PPHSLRVLEE
181 LEGVRRRSFN  DFRSILPSNL  LTVCQEGTLK  RKTSSVSSIS  QVSPERGMP  PSSPGGSFAH
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  SGKKSQPSMY  EQLRDIAIDN  ICRLKAGLT
541 VDPVIVEAFL  ASLSNRLYIS  QESDKDAHLI  PDHTIRALGH  IAVALRDTPK  VMEPIQLIQ
601 QKFCQPPSPL  DVLIIDQLGC  LVITGNQYIY  QEVWNLFQQI  SVKASSVVYS  ATKDYKDHGY
661 RHCSLAVINA  LANIAANIQD  EHLVDELLMN  LLELFVQLGL  EGKRASERAS  EKGPAKASS
721 SAGNLGVLIP  VIAVLRRLP  PIKEAKPRLQ  KLFDRDFWLY  VLMGFAVEGS  GLWPEEWYEG
781 VCEIATKSPL  LTFPSKEPLR  SVLQYNSAMK  NDTVTPAELS  ELRSTIINLL  DPPPEVSALI
841 NKLDFAMSTY  LLSVYRLEYM  RVLRSTDPDR  FQVMFCYFED  KAIQKDKSGM  MQCVIAVADK
901 VFDAFLNMMA  DKAKTKENEE  ELERHAQFLL  VNFNHIHKRI  RRVADKYLGS  LVDKFPHLLW
961 SGTVLKTMLD  ILQTL SLSLS  ADIHKDQPYY  DIPDAPYRIT  VPDTYEARES  IVKDF AARCG
1021 MILQEAMKWA  PTVTKSHLQE  YLNKHQNWVS  GLSQHTGLAM  ATE SILHFAG  YNKQNTT LGA
1081 TQLSERPACV  KKDYSNFMAS  LNLNRNYAGE  VYGMIRFSGT  TGQMSDLNKM  MVQDLHSALD
1141 RSH PQHYTQA  MFKLTAMLIS  SKDCDPQLLH  HLCWGPLRMF  NEHGMETALA  CWEWLLAGKD
1201 GVEVPFMREM  AGAWHMTVEQ  KFGLFSAEIK  EADPLAASEA  SQPKPCPEV  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  RSFPPDAILF  YIPQIVQALR
1621 YDKMGYVREY  ILWAASKSQL  LAHQFIWNMK  TNIYLDEEGH  QKDPDIGDLL  DQLVEEITGS
1681 LSGPAKDFYQ  REFDFFNKIT  NVSAIIPYP  KGDERKACL  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 $\alpha$ nucleotide sequence:

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1  atgggaggtg  actacaaaga  cgatgacgac  aaggcgcgcg  ctctttgcat  gtgtccagtg
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121  atttttctga  ttgaatctga  tcttcagcac  aaagattgtg  tggttcctta  ccttcttcga
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6181 tga

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