Certificate of Analysis
PIK3C3 (hVPS34), 20 µg

Phosphoinositide-3-Kinase, Class 3, GST-tagged

Part Number: PV5126
Lot Number: 853378D
Immediate Storage: -80°C
Shipping Conditions: dry ice

5791 Van Allen Way
Carlsbad, CA 92008
Phone: 760.603.7200
Fax: 760.602.6500
www.lifetechnologies.com

Description:

Recombinant human full-length protein, GST-tagged, expressed in insect cells. No special measures were taken to activate this kinase.

Specific Activity:

23 nmoles of ADP formed per minute per mg of total protein using the Adapta® Assay (PV5099). An ADP/ATP standard curve is used to calculate percent conversion of ATP to ADP. PIK3C3 (hVPS34) activity determined at a final protein concentration of 156 ng/mL.

Concentration:

0.94 mg/mL total protein as measured using the Bradford protein assay with BSA as a standard.

Calculated **7,290 nM**.

Aliases:

Vps34, hVps34, hsVps34, MGC61518

Storage and Handling:

For maximum recovery please spin prior to use. Aliquots of the 5 ug, 10ug and 20ug sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 20 µL are not recommended. **Please never store a kinase diluted.** If properly stored at −80°C, this product is guaranteed for 6 months from date of purchase.

Storage Buffer:

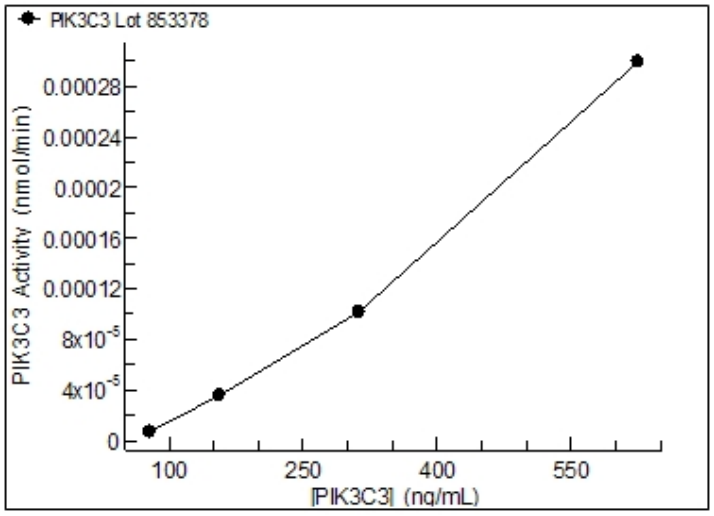
50 mM Tris (pH 7.5), 150 mM NaCl, 0.5 mM EDTA, 0.02% CHAPS, 2 mM DTT and 50% Glycerol.

Assay Buffer:

50 mM HEPES (pH 7.5), 2 mM $MnCl_2$, 0.1% CHAPS, 1 mM EGTA and 2 mM DTT prepared from 5X Kinase Buffer Q (PV5125) with $MnCl_2$ and DTT added.

QUALITY ASSURANCE

PIK3C3 (hVPS34) Activity Graph



Assay Conditions:

PIK3C3 (hVPS34) was diluted in assay buffer and the activity determined in a 10 µL Adapta® Assay (PV5099) with 10 µM ATP and 100 µM PI:PS Lipid Kinase Substrate (PV5122) at room temperature for 1 hour. The reaction was stopped with a 5 µL addition of EDTA and detection reagents. The final concentrations are 10 mM EDTA, 2 nM Adapta® Eu-anti-ADP Antibody and 10 nM Alexa Fluor® 647 ADP Tracer. The plate was read after a 45 minute room temperature incubation.

Gel Information for PIK3C3 (hVPS34)

Page Description: The SDS-PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex® gels (Catalog #: EC6025BOX).

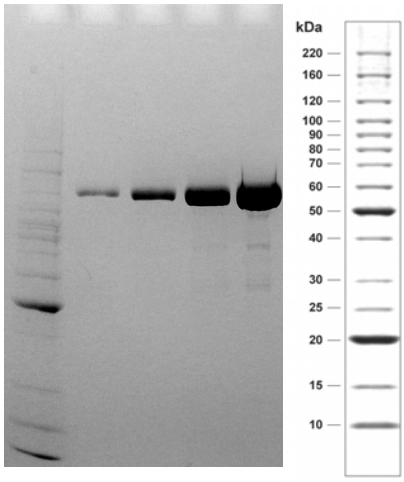
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 1 µg PIK3C3 (hVPS34)

Lane 3: 2 µg PIK3C3 (hVPS34)

Lane 4: 5 µg PIK3C3 (hVPS34)

Lane 5: 10 µg PIK3C3 (hVPS34)



Purity:

95% as determined by a Coomassie® blue stained SDS-PAGE gel.

Molecular Weight:

129.0 kDa calculated from the protein sequence(s). Calculated from the protein sequence(s).

Mass Spectrometry:

PIK3C3 (hVPS34) was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified PIK3C3 (hVPS34) identity by comparison against the amino acid sequence(s) of the recombinant protein.

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_002638.2

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   1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL GST TAG
   1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL IVGN PIK3C3
   1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- NP_002638.2

 101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
 101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
   1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 201 WPLQGWQATF GGGDHPPKSD LVPR
 201 WPLQGWQATF GGGDHPPKSD LVPRHNQTSL YKKAGTMGEA EKFHYIYSCD LDINVQLKIG SLEGKREQKS YKAVLEDPML KFSGLYQETC SDLYVTCQVF
   1 ---------- ---------- ---------- ------MGEA EKFHYIYSCD LDINVQLKIG SLEGKREQKS YKAVLEDPML KFSGLYQETC SDLYVTCQVF

 224
 301 AEGKPLALPV RTSYKAFSTR WNWNEWLKLP VKYPDLPRNA QVALTIWDVY GPGKAVPVGG TTVSLFGKYG MFRQGMHDLK VWPNVEADGS EPTKTPGRTS
  65 AEGKPLALPV RTSYKAFSTR WNWNEWLKLP VKYPDLPRNA QVALTIWDVY GPGKAVPVGG TTVSLFGKYG MFRQGMHDLK VWPNVEADGS EPTKTPGRTS

 224
 401 STLSEDQMSR LAKLTKAHRQ GHMVKVDWLD RLTFREIEMI NESEKRSSNF MYLMVEFRCV KCDDKEYGIV YYEKDGDESS PILTSFELVK VPDPQMSMEN
 165 STLSEDQMSR LAKLTKAHRQ GHMVKVDWLD RLTFREIEMI NESEKRSSNF MYLMVEFRCV KCDDKEYGIV YYEKDGDESS PILTSFELVK VPDPQMSMEN

 224
 501 LVESKHHKLA RSLRSGPSDH DLKPNAATRD QLNIIVSYPP TKQLTYEEQD LVWKFRYYLT NQEKALTKFL KCVNWDLPQE AKQALELLGK WKPMDVEDSL
 265 LVESKHHKLA RSLRSGPSDH DLKPNAATRD QLNIIVSYPP TKQLTYEEQD LVWKFRYYLT NQEKALTKFL KCVNWDLPQE AKQALELLGK WKPMDVEDSL

 224
 601 ELLSSHYTNP TVRRYAVARL RQADDEDLLM YLLQLVQALK YENFDDIKNG LEPTKKDSQS SVSENVSNSG INSAEIDSSQ IITSPLPSVS SPPPASKTKE
 365 ELLSSHYTNP TVRRYAVARL RQADDEDLLM YLLQLVQALK YENFDDIKNG LEPTKKDSQS SVSENVSNSG INSAEIDSSQ IITSPLPSVS SPPPASKTKE

 224
 701 VPDGENLEQD LCTFLISRAC KNSTLANYLY WYVIVECEDQ DTQQRDPKTH EMYLNVMRRF SQALLKGDKS VRVMRSLLAA QQTFVDRLVH LMKAVQRESG
 465 VPDGENLEQD LCTFLISRAC KNSTLANYLY WYVIVECEDQ DTQQRDPKTH EMYLNVMRRF SQALLKGDKS VRVMRSLLAA QQTFVDRLVH LMKAVQRESG

 224
 801 NRKKKNERLQ ALLGDNEKMN LSDVELIPLP LEPQVKIRGI IPETATLFKS ALMPAQLFFK TEDGGKYPVI FKHGDDLRQD QLILQIISLM DKLLRKENLD
 565 NRKKKNERLQ ALLGDNEKMN LSDVELIPLP LEPQVKIRGI IPETATLFKS ALMPAQLFFK TEDGGKYPVI FKHGDDLRQD QLILQIISLM DKLLRKENLD

 224
 901 LKLTPYKVLA TSTKHGFMQF IQSVPVAEVL DTEGSIQNFF RKYAPSENGP NGISAEVMDT YVKSCAGYCV ITYILGVGDR HLDNLLLTKT GKLFHIDFGY
 665 LKLTPYKVLA TSTKHGFMQF IQSVPVAEVL DTEGSIQNFF RKYAPSENGP NGISAEVMDT YVKSCAGYCV ITYILGVGDR HLDNLLLTKT GKLFHIDFGY

 224
1001 ILGRDPKPLP PPMKLNKEMV EGMGGTQSEQ YQEFRKQCYT AFLHLRRYSN LILNLFSLMV DANIPDIALE PDKTVKKVQD KFRLDLSDEE AVHYMQSLID
 765 ILGRDPKPLP PPMKLNKEMV EGMGGTQSEQ YQEFRKQCYT AFLHLRRYSN LILNLFSLMV DANIPDIALE PDKTVKKVQD KFRLDLSDEE AVHYMQSLID

 224
1101 ESVHALFAAV VEQIHKFAQY WRK
 865 ESVHALFAAV VEQIHKFAQY WRK
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* highlighted residues denote differences from the reference protein sequence(s).

Becky. Baker, QA Engineer II Date: 11/Apr/2012