

Certificate of Analysis

ARK5, active

(Recombinant enzyme expressed in Sf21 cells)

Item # 14-661, 14-661-K, 14-661M

Parent Lot # 1654019

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 ARK5, amino acids 2–end expressed by baculovirus in Sf21 cells. Purified using immobilized metal affinity chromatography. Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 78kDa.

Specific Activity (Parent lot# 1654019): 273U/mg, where one unit of ARK5, active activity is defined as 1nmol phosphate incorporated into 300μM (KKKVSRSGLYRSPSPENLNRP) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.489mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

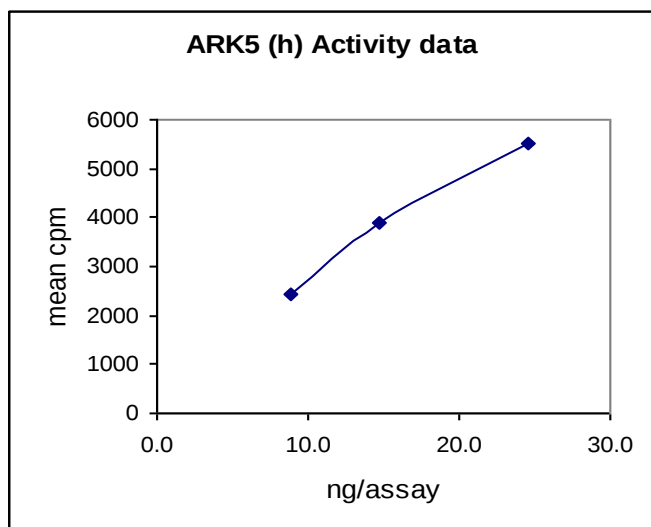
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

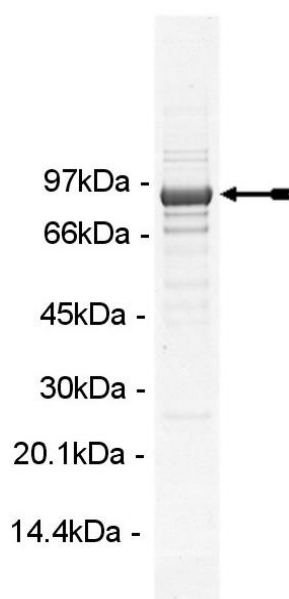
Quality Control Testing

Kinase Assay: 8.9–24.5ng of this lot of enzyme phosphorylated 300μM (KKKVSRSGLYRSPSPENLNRP) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as ARK5 with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3μg of ARK5, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKKVSRSGLYRSPSPENLNRPR):** Use at a final concentration of 300 μ M. Make a 3mM stock. Add 2.5 μ l per assay point.
3. **ARK5, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 8.9–24.5ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKKVSRSGLYRSPSPENLNRPR)**.
3. Add **2.5 μ l (8.9–24.5ng) ARK5, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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ARK5 Sequence Information

<u>Protein</u>	Human ARK5
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	E31 of the recombinant protein is equivalent to E2 of human ARK5
<u>Accession number</u>	GenBank NM_014840

Recombinant ARK5 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF EGAAAPVAGD RPDGLGLGAPG SPREAVAGAT
61 AALEPRKPHG VKRHHHKHNL KHRVELQETL GKGTYGKVKR ATERFSGRVV AIKSIRKDKI
121 KDEQDMVHIR REIEMSSLN HPHIISIYEV FENKDKIVII MEYASKGELY DYISERRRLS
181 ERETRHFFRQ IVSAVHYCHK NGVVRDLKL ENILLDDNCN IKIADFGLSN LYQKDKFLQT
241 FCGSPLYASP EIVNGRPYRG PEVDSWALGV LLYTLVYGTM PFDGFDHKNL IRQISSGEYR
301 EPTQPSDARG LIRWMLMVNP DRRATIEDIA NHWWVNWGYK SSVCDALH DSESPLLARI
361 IDWHHRSTGL QADTEAKMKG LAKPTTSEVM LERQSLKKS KKENDFAQSG QDAVPESPSK
421 LSSKRPKGIL KKRSNSEHRS HSTGFIEGVV GPALPSTFKM EQDLCRTGVL LPSSPEAEVP
481 GKLSPKQSAT MPKKGILKKT QQRESGYYS PERSESELL DSNDVMGSSI PSPSPDPAR
541 VTSLSLSCRR KGILKHSSKY SAGTMDPALV SPEMPTLESL SEPGVPAEGL SRSYSRPSSV
601 ISDDSVLSSD SFDLLDLQEN RPARQIRSC VSAENFLQIQ DFEGQLNRPR PQYLKRYRNR
661 LADSSFSLLT DMDDVTQVYK QALEICSKLN

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Recombinant ARK5 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc gaaggggccc cgcgcctgt ggcgggggac
121 cgccccgact tggggctggg ggcgcggggc tctccccgag aggcgggtggc gggggcgact
181 gcagccctgg agcccaggaa gccgcacggg gtgaagcggc atcaccacaa gcacaacttg
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2041 caagcgctgg agatctgcag caagctcaac tag
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