

ZIPK, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-608, 14-608-K, 14-608M

Parent Lot # 1593245

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, GST tagged, recombinant, of human ZIPK amino acids 1–290, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 56.9% by SDS-PAGE and Coomassie blue staining. MW = 64.3kDa.

Specific Activity (Parent lot# 1593245): 239U/mg, where one unit of ZIPK, active activity is defined as 1nmol phosphate incorporated into 250μM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.361mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

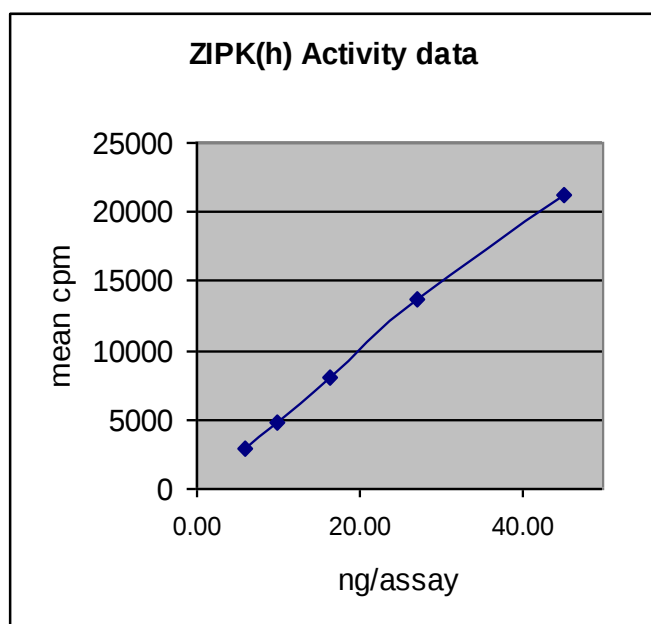
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

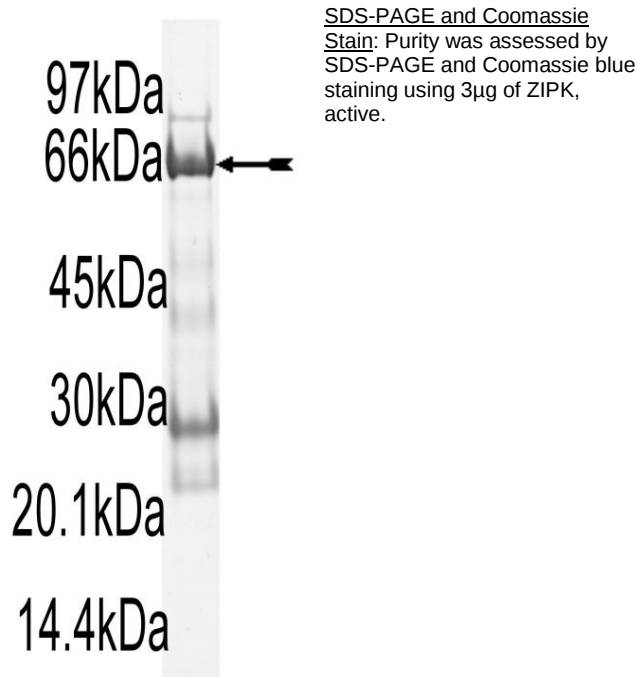
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6–45ng of this lot of enzyme phosphorylated 250μM (KKLNRTLSFAEPG) 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 product identity as ZIPK with the translated native sequence listed on page three.



Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKLNRTLSTFAEPG):** Use at a final concentration of 250 μ M. Make up a 2.5mM stock. Use 2.5 μ l of stock per assay point.
3. **ZIPK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6–45ng 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 **(KKLNRTLSTFAEPG)**.
3. Add **2.5 μ l (6–45ng) ZIPK, 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 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 enzyme components plus 1 μ l 30% phosphoric acid.

ZIPK Sequence Information

Protein	human ZIPK
Tags	N-terminal GST
Native sequence	M266 of the recombinant protein is equivalent to M1 of human ZIPK
Accession number	GenBank AB007144

Recombinant ZIPK amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPEFG  TRGGVAIRGL
241 LRSYLQAAVT  ALSLEWKLE  GGPAAMSTFR  QEDVEDHYEM  GEELGSGQFA  IVRKCRQKGT
301 GKEYAAKFIK  KRRLLSSRRG  VSREEIEREV  NILREIRHPN  IITLHDIFEN  KTDVVLILEL
361 VSGGELFDFL  AEKESLTEDE  ATQFLKQILD  GVHYLHAKRI  AHFDLKPENI  MLLDKNVNP
421 RIKLIDFGIA  HKIEAGNEFK  NIFGTPEFVA  PEIVNYEPLG  LEADMWSIGV  ITYILLSGAS
481 PFLGETKQET  LTNISAVNYD  FDEEYFSNTS  ELAKDFIRRL  LVKDPKRRMT  IAQSLEHSWI
541 KAIRRRNVRG  EDSGRIVTD

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Recombinant ZIPK nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
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1621  aaggcgatcc ggcggcggaa cgtgcgtggt gaggacagcg gccgcacgtg gactgactga
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