

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 vialing runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialing 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.

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.

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.

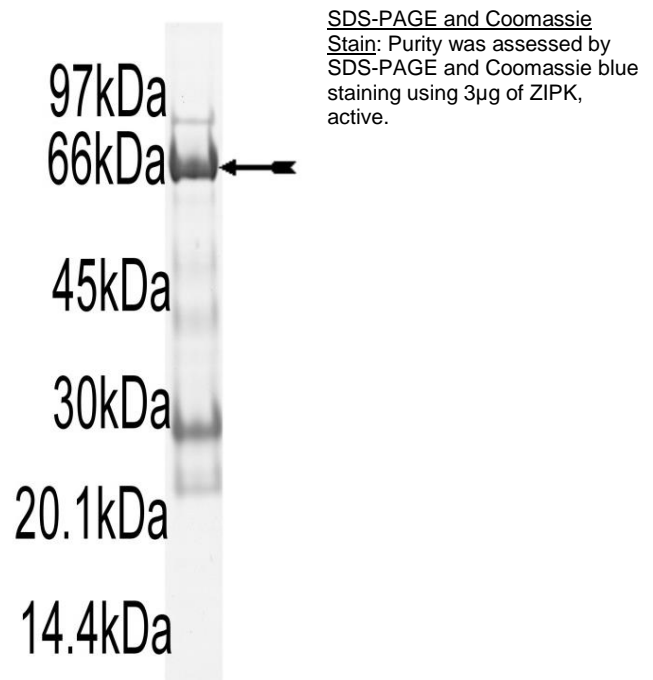
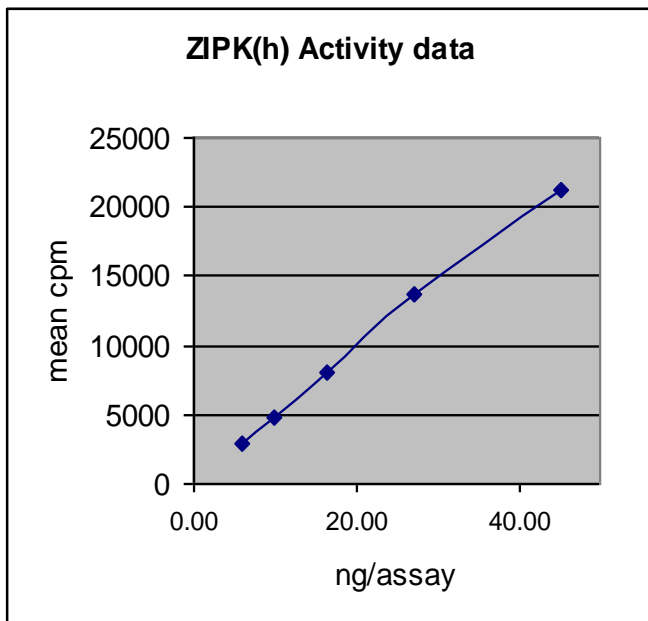
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 ProtocolStock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKLNRTLSEFAEPG):** Use at a final concentration of 250µM. Make up a 2.5mM stock. Use 2.5µl 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKLNRTLSEFAEPG)**.
3. Add **2.5µl (6–45ng) ZIPK, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl 3% phosphoric acid.
8. Transfer a 10µl 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 1µl 30% phosphoric acid.

ZIPK Sequence Information

<u>Protein</u>	human ZIPK
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M266 of the recombinant protein is equivalent to M1 of human ZIPK
<u>Accession number</u>	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  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAI PQID  KYLKSSKYIA  WPLQG WQATF  GGGDHPPKSD  LVPRGSPEFG  TRGGVAIRGL
241  LRSYLQAAVT  ALSLEWKLE  GGPAAMSTFR  QEDVEDHYEM  GEELGSGQFA  IVRKCRQKGT
301  GKEYAAKFIK  KRRLSSSRG  VSREEIEREV  NILREIRHPN  IITLHDIFEN  KTDVVLILEL
361  VSGGELFDFL  AEKESLTEDE  ATQFLKQILD  GVHYLH SKRI  AHFDLKPENI  MLLDKNV PNP
421  RIKLIDFGIA  HKIEAGNEFK  NIFGTPEFVA  PEIVNYEPLG  LEADMWSIGV  ITYILLSGAS
481  PFLGETKQET  LTNISAVNYD  FDEEYFSNTS  ELAKDFIRRL  LVKDPKRRT  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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121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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1141  gccaccagc  tcctcaagca  gatcctggac  ggcgttctact  acctgcactc  taagcgcatac
1201  gcacactttg  acctgaagcc  ggaaaacatc  atcgtgctgg  acaagaacgt  gccaaccca
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