

Certificate of Analysis

ZAK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 15-015, 15-015-K, 15-015M

Parent Lot # 203591

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, human ZAK full length expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
 Purity 62% by SDS-PAGE and Coomassie blue staining. MW = 119kDa.

Specific Activity (Parent lot# 203591): 820U/mg, where one unit of ZAK activity is defined as 1nmol phosphate incorporated into 0.33mg/ml MBP per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.01mg/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.

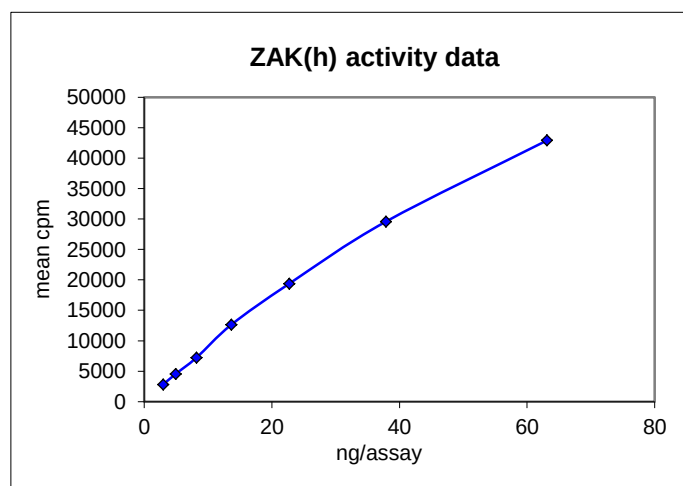
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

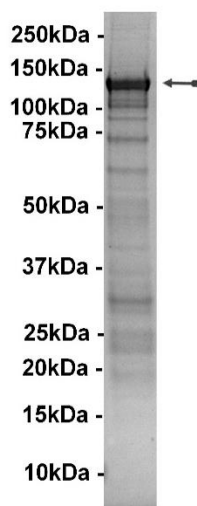
**FOR IN VITRO RESEARCH USE ONLY
 NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3.0–63.1ng of this lot of enzyme phosphorylated 0.33mg/ml MBP 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 ZAK with the translated sequence listed on page three.



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

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Myelin Basic Protein:** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
- 3. ZAK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3.0–63.1ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[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 MBP.
3. Add **2.5µl (3.0–63.1ng) ZAK, 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 of 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 assay components plus 1µl of 30% phosphoric acid.

Certificate of Analysis

ZAK, active Sequence Information

Protein	Human
Tags	N-terminal GST
Native sequence	M236 of the recombinant protein is equivalent to M1 of human ZAK
Accession number	GenBank NM_016653.2 (DNA has been codon optimized for insect cell expression)

Recombinant ZAK amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMa IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFK GLRRRMSSLG
241 ASFVQIKFDD LQFFENC GGG SFGSVYRAKW ISQDKEVAVK KLLKIEKEAE ILSVLSHRNI
301 IQFYGVILEP PNYGIVTEYA SLGSLYDIYN SNRSEEMDMD HIMTWATDVA KGMHYLHMEA
361 PVKVIHRDLK SRNVVIAADG VLKICDFGAS RFHNHTTHMS LVGTFPWWAP EVIQSLPVSE
421 TCDTYSYGVV LWEMLTREVP FKGLEGLQVA WLVEKNERL TIPSSCPRS F AELLHQWEA
481 DAKKRPSFKQ IISILESMN DTSLPDKCNS FLHNKAEWRC EIEATLERLK KLERDLSFKE
541 QELKERERRL KMWEQKLTEQ SNTPLLPSFE IGAWTEDDVY CWVQQLVRKG DSSAEMSVYA
601 SLFKENNITG KRLLLLEED LKDMGIVSKG HIIHFKSAIE KLTHDYINLF HFPPLIKDSG
661 GEPEENEKEI VNLELVFGFH LKPGTGPDQC KWKMYMEMDG DEIAITYIKD VTFNTNLPDA
721 EILKMTKPPF VMEKWIVGIA KSQTVECTVT YESDVRTPKS TKHVHSIQWS RTKPQDEVKA
781 VQLAIQTFLT NSDGNPGSRS DSSADCQWLD TLRMRQIASN TSLQRSQSNP ILGSPFFSHF
841 DGQDSYAAAV RRPQVPIKYQ QITPVNQSR SSSPTQYGLTK NFSSLHLNSR DSGFSSGNTD
901 TSSERGRYS DSRNKYGRGS ISLNSSPRGR YSGKSQHSTP SRGRYPGKFY RVSQSALNPH
961 QSPDFKRSPR DLHQPNITPG MPLHPETDSR ASEEDSKVSE GGWTKVEYRK KPHRPSPAKT
1021 NKERARGDHR GWRNF

```

Recombinant ZAK nucleotide sequence:

```

1 atgtccccta tactaggtta ttgga aaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatt ttgaagaaaa atatgaagag catttgatag agcgcatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcccta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgtaaccat cctgacttca tggtgatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatccaa ggaattcaaa ggcctacgtc gacgaatgag tagccttggc
721 gcctcttttg tacagattaa attcgatgac ctccagttct tcgagaattg cggcgggtgga
781 tctttcggaa gcgtgtacag agctaaatgg atctcccaag acaaagaggt cgcagtcagg
841 aagctcctga aaatcgagaa ggaggccgag atcctgtctg tggtgtcaca tcgcaacatc
901 atccagttct acggagtgat cctggagccc cctaactacg gtatcgtaac ggagtatgca
961 agcctcggta gcttgatga ctatatcaat tctaacaggt cagaggaaat ggatattggc
1021 cacatcatga cttgggcaac cgatgtggcc aaaggcatgc attacctcca catggaggca
1081 cccgtgaaag tgatccatcg cgacttgaaa tctcgcaacg tcgttatcgc tgcagacggc
1141 gttctcaaaa tatgtgattt cggtgcaagt cgcttcaca atcacacaac tcacatgagc

```

Certificate of Analysis

```

1201 ttggtaggaa catttcctg gatggcacc gaagtgate agtccctgcc ggtttccgag
1261 acttgtgaca catactccta cgggtgtgtg ctctgggaga tgctgaccag ggaagtgcc
1321 ttcaaaggat tggagggact ccaggtagcc tggctgggtg tagagaaaaa tgaaaggctg
1381 acaattcctt cctcttgccc caggtccttc gcagagttgt tgcaccagtg ctgggaagct
1441 gacgctaaaa agaggccttc cttcaaacag atcatttcca tactggagtc tatgtctaac
1501 gacacttcct tgcccataa gtgcaactct ttcctgcata ataaggctga atggagatgc
1561 gaaatcgagg ctaccctcga gaggtgaaa aagctcgaga gggaccttc tttcaaggaa
1621 caggaattga aggaacgcga aaggcgctg aagatgtggg agcagaagct cacagagcag
1681 tctaatactc ctcttctgcc aagcttcgag attggagctt ggactgagga cgatgtgtac
1741 tgttgggtcc aacagttggt gaggaaggga gacagttccg ccgagatgtc cgtttatgct
1801 tcaactgtta aggaaaataa cattactggt aagagattgc tcttggtgga agaggaagat
1861 ctgaaggata tgggcatcgt cagcaaaggt cacattattc actttaaatc tgctatagag
1921 aagcttacc acgattatat caactgttc catttcccac cgcttattaa ggattctgga
1981 ggagagccc aggagaacga ggagaagatc gttaatcttg agttggtgtt cggctttcac
2041 ctgaaacccg gcaccggacc ccaggactgc aagtggaaaa tgtatatgga aatggatggt
2101 gatgaaattg ctatcaccta tattaaggat gttaccttca ataccaatct tccagatgcg
2161 gagatcctga agatgaccaa acctcccttc gtcattggaga agtggatcgt cggcatagct
2221 aagtcccaa cgggtggaat cacagtcact tatgagagcg atgtcagaac acccaagtct
2281 actaagcatg tccacagtat ccagtgttcc cgcacaaagc cgcaggatga agttaaggca
2341 gtacagctgg ccatccagac actctttact aactctgacg gtaaccccg ctcagaagt
2401 gattcttccg ccgactgcca gtggctcgac acgttgagaa tgcgccagat tgcgagcaac
2461 accagctgc agaggtcaca gtccaatcct atcttgggca gtcccttct ctctacttt
2521 gacggacagg actcttacgc ggctgctgtc agaaggcccc aagtcccat caagtaccag
2581 cagatcacc cagttaacca aagtgcgtcc tcctccccta cacagtacgg ccttaccaag
2641 aatttttagc gcttgacact caactctgc gacagcggt tctcatccg aaacaccgat
2701 acttctcctg agaggggacg ctactccgac agaagcagga acaagtatg aagaggatca
2761 atcagcctta atagtagccc tagaggcaga tactctggca agtcccagca cagtacacca
2821 agcaggggca ggtaccctgg caagttctat cgcgtttcac agtctgctct gaaccacat
2881 caaagtccag acttcaagcg ctccccgagg gatctgcacc agcccaacac gatccccggc
2941 atgcccctgc accctgaaac cgattctcgc gcttccgagg aagattccaa agttagcgaa
3001 ggagggttga ccaagggtga atatagaaag aagccacaca ggcctagtcc cgccaagaca
3061 aataaggaaa gagccagagg agatcatcgc ggatggagga acttctaa
  
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.