

Certificate of Analysis

ZAP-70, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-404, 14-404-K, 14-404M

Parent Lot # 1624853

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: C-terminal 6His-tagged, recombinant, human, full-length ZAP-70, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 90.8% by SDS-PAGE and Coomassie blue staining. MW = 70.8kDa.

Specific Activity (Parent lot# 1624853): 62U/mg, where one unit of ZAP-70 activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

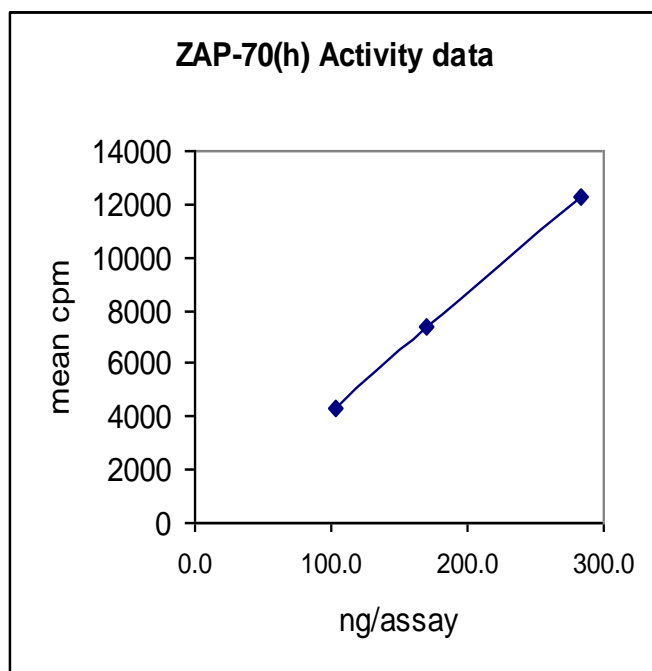
Formulation: 1.134mg/ml of enzyme in of 25mMHepes/NaOH pH7.4, 150mM NaCl, 0.5mM DTT, 50% glycerol. Liquid at -20°C.

Storage and Stability: On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 6 months 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.

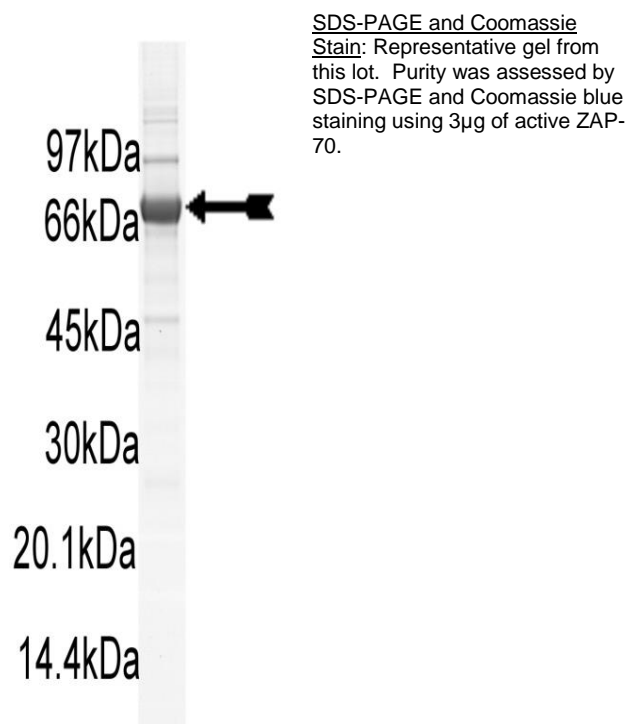
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 103–284ng of this lot of enzyme phosphorylated 0.1 mg/ml poly(Glu,Tyr) (4:1) 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 human ZAP-70 with the translated native sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

- 10 x Reaction Buffer:** 500mM Tris/HCl pH 7.5, 1mM EGTA, 1% 2-mercaptoethanol, 1mM Na₃VO₄.
- Poly(Glu,Tyr) (4:1):** use a final assay concentration of 0.1mg/ml. Make up a 1mg/ml stock. Use 2.5µl of stock per assay point.
- ZAP-70, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.1mM Na₃VO₄, 1mg/ml BSA. Use 103–284ng per assay point.
- [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 2.5µl of 10 x reaction buffer to wells.
2. Add 2.5µl of **poly(Glu,Tyr) (4:1)**.
3. Add 2.5µl (**103–284ng**) **ZAP-70, active**.
4. Add 7.5µl 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 **Filtermat A**.
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

ZAP-70 Sequence Information

<u>Protein</u>	Human ZAP-70
<u>Tags</u>	C-terminal 6His
<u>Native sequence</u>	M1 of the recombinant protein is equivalent to M1 of human ZAP-70
<u>Accession number</u>	GenBank L05148

Recombinant ZAP-70 amino acid sequence:

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1 MPDPAHLPF FYGSISRAEA EEHLKLAGMA DGLFLLRQCL RSLGGYVLSL VHDVRFHHFP
61 IERQLNGTYA IAGGKAHCGP AELCEFYSRD PDGLPCNLRK PCNRPSGLEP QPGVFDCLRD
121 AMVRDYVRQT WKLEGEALEQ AIISQAPQVE KLIATTAHER MPWYHSSLTR EEAERKLYSG
181 AQTGDKFLLR PRKEQGTAL SLIYGKTVYH YLISQDKAGK YCIPEGTKFD TLWQLVEYLK
241 LKADGLIYCL KEACPSSAS NASGAAAPTL PAHPSTLTHP QRRIDTLNSD GYTPEPARIT
301 SPDKPRPMPM DTSVYESPYS DPEELKDKKL FLKRDNLLIA DIELGCGNFG SVRQGVYRMR
361 KKQIDVAIKV LKQGTEKADT EEMMREAQIM HQLDNPYIVR LIGVCQAEAL MLVMEMAGGG
421 PLHKFLVGKR EEIPVSNVAE LLHQVSMGMK YLEEKNFVHR DLAARNVLLV NRHYAKISDF
481 GLSKALGADD SYYTARSAGK WPLKWYAPEC INFRKFSSRS DVWSYGVTMW EALSYGQKPY
541 KKMKGPEVMA FIEQKRMED PPECPPELYA LMSDCWIYKW EDRPDFLTVE QRMRACTYSL
601 ASKVEGPPGS TQKAEACAAH HHHHH
  
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Recombinant ZAP-70 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatadc ttgaagaaaa atatgaagag catttgcgatg agcgcgatga aggtgataaa
121 tggcgaagaa aaaagtttga attgggtttg gagtttcca atcttcctta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
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1741 ggtattatac accgtgactt aaagccagag aatgttttac tgtcatctca agaagaggac
  
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Certificate of Analysis

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1801 tgtcttataa agattactga ttttgggcac tccaagattt tgggagagac ctctctcatg
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2161 agacacccgt ggcttcagga tgaagacatg aagagaaagt ttcaagatct tctgtctgag
2221 gaaaatgaat ccacagctct accccagggt ctagcccagc cttctactag tcgaaagcgg
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2341 gtgttgcata accatcacca tcactga
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