

Phospho ZAP70 (Y319) Antibody

Rabbit Polyclonal

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|---------------------------|------------|-------------|
| Antigen Affinity Purified | Protein ID | NP_001070.2 |
| Catalog No. A302-007A | GeneID | 7535 |
| Lot No. A302-007A-1 | | |



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|------------------------------|--|
| APPLICATIONS | IP, F |
| SPECIES REACTIVITY | Human |
| PRESUMED REACTIVITY | Based on 100% sequence identity, this antibody is predicted to react with Mouse |
| AMOUNT | 100 µl |
| CONCENTRATION | 1000 µg/ml |
| STORAGE/SHELF LIFE | 2 – 8° C / 1 year from date of receipt |
| PHYSICAL STATE | Liquid |
| BUFFER | Tris-citrate/phosphate buffer, pH 7 to 8 containing 0.09% Sodium Azide |
| ISOTYPE | IgG |
| ORIGIN | USA |
| PRODUCTION PROCEDURES | Antibody was affinity purified using an epitope specific to Phospho ZAP70 (Y319) immobilized on solid support. |

The epitope recognized by A302-007A maps to a region including tyrosine 319 (when it is phosphorylated) of human zeta-chain (TCR) associated protein kinase 70kDa using the numbering given in entry NP_001070.2 (GeneID 7535).

Immunoglobulin concentration was determined by extinction coefficient: absorbance at 280 nm of 1.4 equals 1.0 mg of IgG.

APPLICATIONS Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use.

Western Blot Not recommended. A302-007A has not performed satisfactorily when used for WB of phospho ZAP70 in crude preparations (e.g. whole cell lysate). This antibody can be used for WB of enriched (e.g. immunoprecipitated) sources of ZAP70.

Immunoprecipitation 2 – 5 µg/mg lysate

Flow Cytometry 1.0 µg per 1 X 10⁶ cells in a 150 µl volume

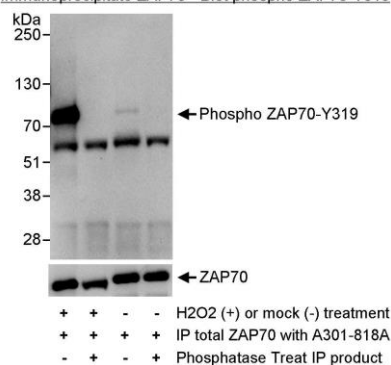
APPLICATION NOTES Western blot of immunoprecipitates performed using Normal Pig Serum (Cat. No. S100-020), Goat anti-Rabbit Light Chain HRP Conjugate (Cat. No. A120-113P) and 4-8% SDS-PAGE (link to IP-western blot protocol in Additional Info section below).

ADDITIONAL INFO <https://www.bethyl.com/product/A302-007A>

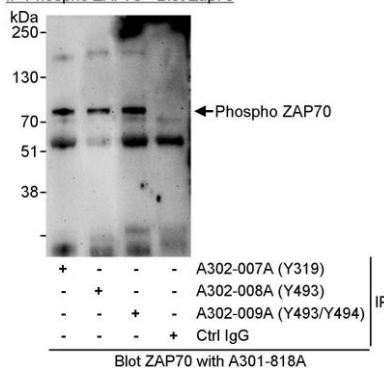
Use the link above to view SDS, a current list of citations, and other product specific information. IP-western blot protocol: https://www.bethyl.com/content/protocol_IP_WB

This document certifies that this product has met all of the quality control standards defined by Bethyl Laboratories, Inc.
Eric McIntush, PhD | Chief Scientific Officer Date: June 21, 2019

Immunoprecipitate ZAP70 - Blot phospho ZAP70-Y319

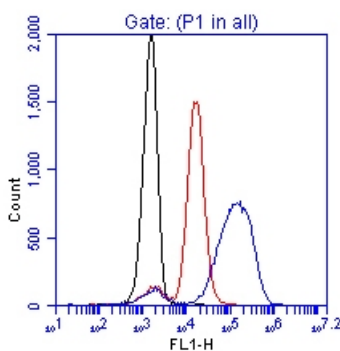


IP Phospho ZAP70 - Blot Zap70



Detection of Phosphorylation of human ZAP70 on Y319 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1 mg for IP, 20% of IP loaded) from Jurkat cells that had been treated with hydrogen peroxide (+) or mock treated (-). *Antibodies:* ZAP70 antibody A301-818A was used at 3 $\mu\text{g}/\text{mg}$ lysate to immunoprecipitate ZAP70. The immunoprecipitates were treated with phosphatase (+) or mock (-) treated. For blotting immunoprecipitated Phospho ZAP70, Phospho ZAP70 (Y319) antibody A302-007A was used at 1 $\mu\text{g}/\text{ml}$. *Detection:* Chemiluminescence with an exposure time of 30 seconds.

Detection of Phosphorylation of human ZAP70 on Y319, Y493 or Y493/Y494 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1 mg for IP, 20% of IP loaded) from Jurkat cells that had been treated with hydrogen peroxide. *Antibodies:* Phospho ZAP70 antibodies A302-007A (phosphorylation at Y319), A302-008A (phosphorylation at Y493), and A302-009A (phosphorylation at Y493/Y494) used for IP at 3 $\mu\text{g}/\text{mg}$ lysate. For blotting immunoprecipitated Phospho ZAP70, ZAP70 antibody A301-818A was used at 1 $\mu\text{g}/\text{ml}$. *Detection:* Chemiluminescence with an exposure time of 3 min.



Flow cytometric analysis of phospho-Zap70 (Y319). Jurkat cells were treated with H2O2 for 3 minutes. Cells were fixed in 1.5% PFA, permeabilized in 90%, and 1 X 10⁶ cells stained with 1.0 $\mu\text{g}/\text{ml}$ Phospho Zap70 (Y319) antibody A302-007A in a 150 μl volume. Isotype anti-KLH control (black) in H2O2 treated cells, anti-phospho Zap70 (Y319) in untreated cells (red), anti-phospho Zap70 (Y319) in H2O2 treated (blue).