

# ZAP-70 [pY315/pY319] ABfinity™ Recombinant



## Rabbit Monoclonal Antibody - Purified

**REF** Catalog no. 700177

(See product label for lot information)

**Clone/PAD:** 4H16L19  
**Isotype:** IgG  
**Gene ID:** 7535  
**Protein Acc. No.:** P43403  
**Qty:** 100 µg  
**Volume:** 200 µl  
**Concentration:** 0.5 mg/ml

### Formulation

PBS + 0.09% sodium azide

### Immunogen

A peptide corresponding to amino acids 311-323 of P43403.

### Immunogen sequence

DTSV[pY]ESP[pY]SDPE

### Reactivity

This antibody reacts with Human ZAP-70 [pY315/pY319]. Based on sequence similarity, reactivity to orangutan, chimpanzee, Rhesus monkey, bovine, and Xenopus is expected.

### Specificity

This antibody is specific for pY315/pY319 and does not recognize non-phosphorylated ZAP-70 protein.

### Storage

2-8°C for up to 1 mo, -20°C for long term storage. Avoid repeated freezing and thawing.



### Expiration Date

Expires one year from date of receipt when stored as instructed.

### Validated Applications:

	Species	Test Material	Concentration
<b>Western Blotting</b>	human	Jurkat	0.5-2 µg/ml
<b>Immunofluorescence</b>	human	Jurkat	1-3 µg/ml
<b>Flow Cytometry</b>	human	Jurkat	1-3 µg/test

### Background

Zeta-associated protein (ZAP-70), a 70 kDa member of the Syk tyrosine kinase family, plays a central role in lymphocyte activation and development, and is implicated in several immune disorders (1-3). Upon T-cell antigen receptor (TCR) engagement, ZAP-70 is phosphorylated on tyrosines 292, 315 and 319 in the interdomain B, located between the SH2 and kinase domains (4,5). Phosphorylation of both tyrosines 315 (a Vav-binding site) and 319 (a Lck binding site) enhances ZAP-70 function in mediating lymphocyte signaling, while tyrosine 292 terminates the transient activation of ZAP-70 and attenuates lymphocyte signaling (6,7). Phosphorylation of tyrosines 315 and 319 plays an important role in mediating the positive and negative selection of T cells in thymus (8). TLR9-activated B-cells display elevated ZAP-70 expression levels which correlate with sustained PKB induction (9). Additionally, (-)-epigallocatechin gallate (EGCG), a major catechin found in green tea, binds directly to ZAP-70 resulting in caspase-mediated apoptosis in Jurkat cells (10).

### References

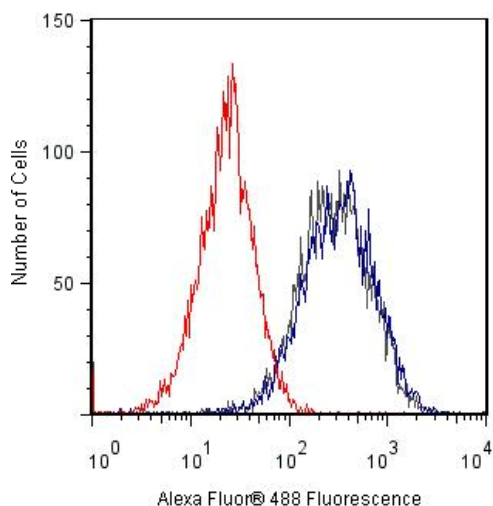
1. Orchard, J.A., et al. (2004) ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. *Lancet* 363: 105-111.
2. Sakaguchi, N., et al. (2003) Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice *Nature* 426: 454-460.
3. Bottini, N., et al. (2002) Activation of ZAP-70 through specific dephosphorylation at the inhibitory Tyr-292 by the low molecular weight phosphotyrosine phosphatase (LMPTP). *J. Biol. Chem.* 277: 24220-24224.
4. Di Bartolo, V., et al. (2002) Tyrosine 315 determines optimal recruitment of ZAP-70 to the T cell antigen receptor. *Eur. J. Immunol.* 32: 568-575.
5. Magnan, A., et al. (2001) T cell development and T cell responses in mice with mutations affecting tyrosines 292 or 315 of the ZAP-70 protein tyrosine kinase. *J. Exp. Med.* 194: 491-505.
6. Gong, Q., et al. (2001) Requirement for tyrosine residues 315 and 319 within zeta chain-associated protein 70 for T cell development. *J. Exp. Med.* 194: 507-518.
7. Wu, J., et al. (1997) The Vav binding site (Y315) in ZAP-70 is critical for antigen receptor-mediated signal transduction. *J. Exp. Med.* 185: 1877-1882.
8. Kong, G., et al. (1996) Distinct tyrosine phosphorylation sites in ZAP-70 mediate activation and negative regulation of antigen receptor function. *Mol. Cell. Biol.* 16: 5026-5035.
9. Bekereldjian-Ding, I. et al. (2008) TLR9-activating DNA up-regulates ZAP70 via sustained PKB induction in IgM<sup>+</sup> B cells. *J. Immunol.* 181: 8267-8277.
10. Shim, J-H. et al. (2008) (-)-Epigallocatechin gallate regulates CD3-mediated T cell receptor signaling in Leukemia through the inhibition of ZAP-70 kinase. *J. Biol. Chem.* 283: 28370-28379.

**For research use only. CAUTION: Not intended for human or animal therapeutic or diagnostic use.**

[www.invitrogen.com](http://www.invitrogen.com)

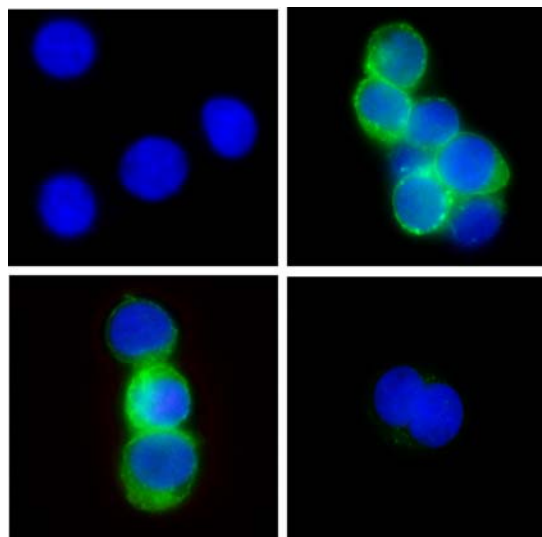
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.



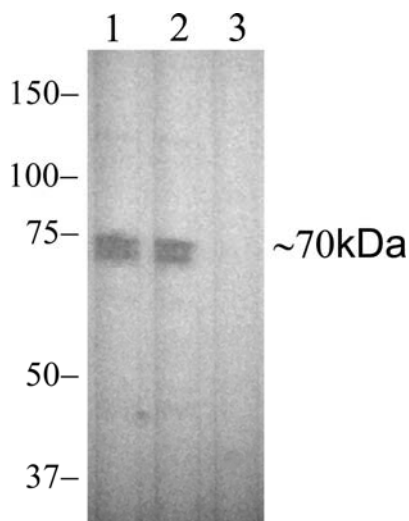
**Flow cytometry of Jurkat cells labeled with rabbit anti-ZAP-70 [pY315/pY319] (Cat. No. 700177).**

Jurkat cells were fixed and permeabilized using FIX & PERM® reagents (Cat. No. GAS004). Cells were then stained with 2 µg/test anti-ZAP-70 [pY315/pY319] followed by Alexa Fluor® 488 goat anti-rabbit Ig [Cat. No. A11008] (gray trace). Pre-incubation with the phosphopeptide used as an immunogen decreased the signal (red trace) while pre-incubation with non-phosphopeptide had no effect (blue trace). Note that blue and gray traces are nearly identical.



**Immunocytochemistry of Jurkat cells labeled with rabbit anti-ZAP-70 [pY315/pY319] (Cat. No. 700177).**

Jurkat cells were either untreated (top left) or treated with H<sub>2</sub>O<sub>2</sub> (top right, bottom) and labeled with rabbit anti-ZAP-70 [pY315/pY319] (2 µg/ml). Alexa Fluor® 488 goat anti-rabbit (Cat. No. A11008) at 1:1000 was used as secondary antibody. Pre-incubation of treated cells with phosphopeptide immunogen decreased signal (bottom right) while pre-incubation with non-phosphopeptide did not (bottom right). Nucleus is stained with Hoescht (blue), AF488 signal (ZAP-70 [pY315/pY319], green).



**Western blot of Jurkat lysates labeled with rabbit anti-ZAP-70 [pY315/pY319] (Cat. No. 700177).**

Rabbit anti-ZAP-70 [pY315/pY319] (1 µg/ml) was used to label ZAP-70 [pY315/pY319] in serum starved, H<sub>2</sub>O<sub>2</sub> treated Jurkat lysates. Pre-incubation with the phosphopeptide used as an immunogen eliminated the signal (lane 3) whereas pre-incubation with the non-phosphopeptide did not (lane 2).

**For research use only. CAUTION: Not intended for human or animal therapeutic or diagnostic use.**

[www.invitrogen.com](http://www.invitrogen.com)

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.