

# Phospho-Zap-70 (Tyr319)/Syk (Tyr352) Antibody

- Small 100  $\mu$ l  
(10 western blots)
- Large 300  $\mu$ l  
(30 western blots)

rev. 08/23/10

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

- Orders** ■ 877-616-CELL (2355)  
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Entrez-Gene ID # 7535  
Swiss-Prot Acc. # P43403

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**\*Species cross-reactivity is determined by western blot.**

**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

### Recommended Antibody Dilutions:

Western Blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (IF-IC)	1:50
Flow Cytometry	1:25

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

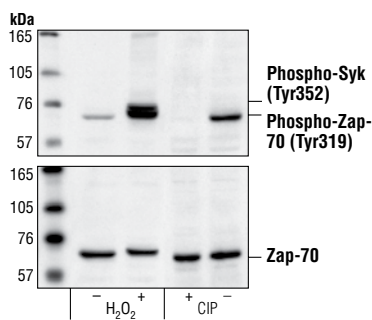
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

Applications W, IP, IF-IC, F Endogenous	Species Cross-Reactivity*		Molecular Wt. 70 kDa Zap-70, 72 kDa Syk	Source Rabbit**
	H	M		

**Background:** Zap-70, a Syk family protein tyrosine kinase expressed in T and NK cells, plays a critical role in mediating T cell activation in response to T cell receptor (TCR) engagement (1). Following TCR engagement, Zap-70 is rapidly phosphorylated on several tyrosine residues, presumably by two mechanisms: autophosphorylation and transphosphorylation by the Src family tyrosine kinase, Lck (2-6). Tyrosine phosphorylation of Zap-70 correlates with its increased kinase activity and downstream signaling events. In patients with chronic lymphocytic leukemia (CLL), total Zap-70 expression was shown to be correlated with disease progression and survival (7,8).

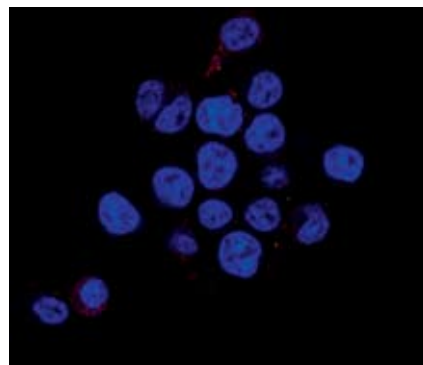
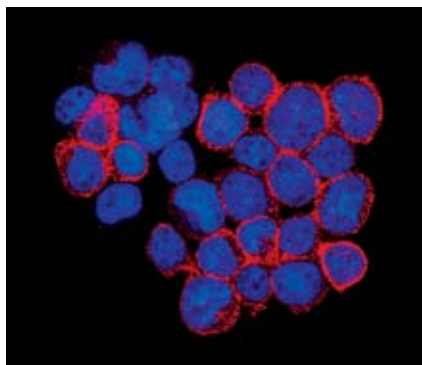
Phosphorylation of Tyr319 is required for the assembly of a Zap-70-containing signaling complex that leads to the activation of the PLC- $\gamma$ 1-dependent and Ras-dependent signaling cascades in antigen-stimulated T cells (5,6). The orthologous Tyr352 residue in Syk is also involved in the association with PLC- $\gamma$ 1 (9).

**Specificity/Sensitivity:** Phospho-Zap-70 (Tyr319)/Syk (Tyr352) Antibody detects endogenous levels of Zap-70 only when phosphorylated at Tyr319. It cross-reacts with endogenous levels of Syk when phosphorylated at Tyr352.



Western blot analysis of extracts from Jurkat cells, starved for 16 hours, and treated with 2 mM H<sub>2</sub>O<sub>2</sub> or with calf intestinal alkaline phosphatase (CIP), using Phospho-Zap-70 (Tyr319)/Syk (Tyr352) Antibody (upper) or control Zap-70 Antibody #2702 (lower).

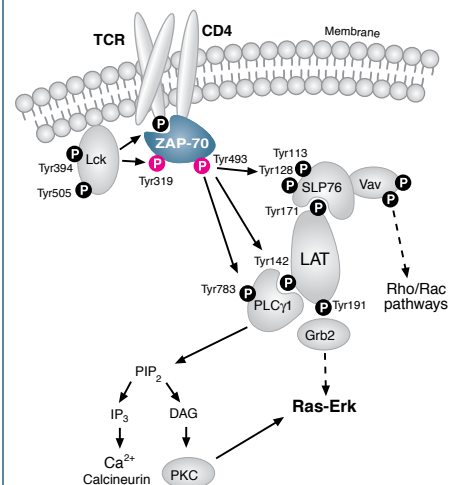
**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr319 of human Zap-70. Antibodies are purified by protein A and peptide affinity chromatography.



Immunofluorescent analysis of Jurkat cells, CD3-treated (left) or untreated (right), using Phospho-Zap-70 (Tyr319)/Syk (Tyr352) Antibody (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

**IMPORTANT:** For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

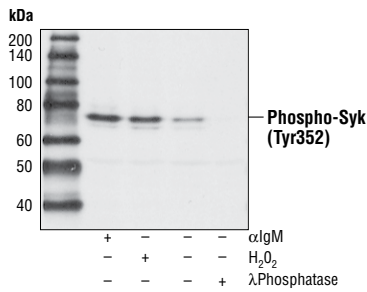
DRAQ5® is a registered trademark of Biostatus Limited.



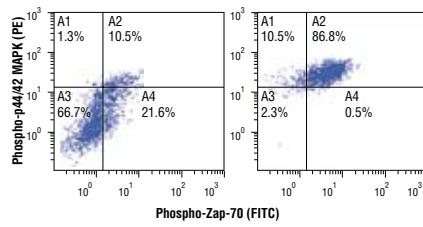
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

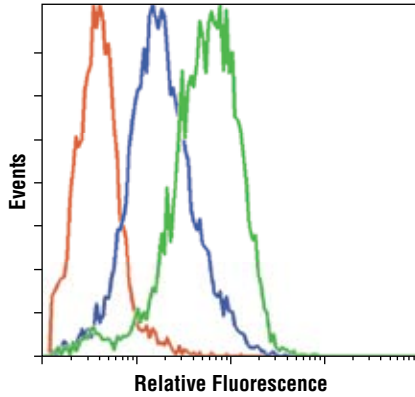
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of extracts from Ramos cells, untreated or treated with anti-IgM (12  $\mu$ g/ml for 2 minutes), hydrogen peroxide (10 mM for 2 minutes) or  $\lambda$  phosphatase, using Phospho-Zap-70 (Tyr319)/ Syk (Tyr352) Antibody.



Two-color flow cytometric analysis of Jurkat cells, untreated (left) or anti-CD3 activated (right), using Phospho-Zap-70 (Tyr319)/Syk (Tyr352) Antibody and Phospho-p44/42 MAPK (Thr202/Tyr204) (E10) mAb #9106. Anti-CD3 activation increases the intensity of label with both antibodies.



Flow cytometric analysis of Jurkat cells, untreated (blue) or CD3 treated (green), using Phospho-Zap70 (Tyr319)/Syk (Tyr352) Antibody compared to a nonspecific negative control antibody (red).

#### Background References:

- (1) Chu, D.H. et al. (1998) *Immunol. Rev.* 165, 167–180.
- (2) Iwashima, M. et al. (1994) *Science* 263, 1136–1139.
- (3) Neumeister, E.N. et al. (1995) *Mol. Cell Biol.* 15, 3171–3178.
- (4) Chan, A.C. et al. (1995) *EMBO J.* 14, 2499–2508.
- (5) Williams, B.L. et al. (1999) *EMBO J.* 18, 1832–1844.
- (6) Di Bartolo, V. et al. (1999) *J. Biol. Chem.* 274, 6285–6294.
- (7) Wiestner, A. et al. (2003) *Blood* 101, 4944–4951.
- (8) Crespo, M. et al. (2003) *N. Engl. J. Med.* 348, 1764–1775.
- (9) Law, C.L. et al. (1996) *Mol. Cell Biol.* 16, 1305–1315.