## **Product Data Sheet**

## Alexa Fluor<sup>™</sup> 647 anti-ZAP70 Phospho (Tyr319)/Syk Phospho (Tyr352)

Catalog # / Size:	4018525 / 25 tests 4018530 / 100 tests
Clone:	1503310
Isotype:	Mouse IgG1, к
Immunogen:	Modified peptide.
<b>Reactivity:</b>	Human
Preparation:	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 647 under optimal conditions.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).
Concentration:	Lot-specific



Human peripheral blood lymphocytes were treated with (top), or without (bottom) H<sub>2</sub>O<sub>2</sub> for five minutes, fixed with Fixation Buffer, permeabilized with True-Phos<sup>™</sup> Perm Buffer, then surface stained with CD3 Brilliant Violet 421&tr

## **Applications:**

Applications:	Flow Cytometry
Recommended	Each lot of this antibody is quality
Usage:	control tested by immunofluoresce

control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 microL per million cells or 5 microL per 100 microL of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.

\* Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633 nm / 635 nm.



**Description:** ZAP70 was identified in TCR-stimulated Jurkat cells. It is an inactive cytosolic tyrosine kinase that is recruited to a transmembrane receptor lacking intrinsic catalytic activity. ZAP70 and the related spleen tyrosine kinase (Syk) play a critical role in T-cell development and activation. This enzyme, which is phosphorylated on tyrosine residues upon T-cell antigen receptor (TCR) stimulation, functions in the initial step of TCR-mediated signal transduction in combination with the Src family kinases, Lck and Fyn.

ZAP70 activation can be regulated by binding to phosphorylated ITAMs of the TCR and by phosphorylation of multiple tyrosine residues on ZAP70.

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Phosphorylation of Tyr315 and Tyr319 are essential for ZAP70 positive regulation of T-lymphocyte activation whereas Tyr292 has a negative regulatory role.

Phosphorylated Tyr319 is a positive regulator of ZAP70, which triggers the binding of Lck and activation of NFAT and IL-2 induction. The binding of Lck to phosphorylated Tyr319 promotes Lck mediated phosphorylation of Tyr493, and facilitates activation of downstream signaling. The importance of Tyr319 phosphorylation in positive regulation of ZAP70 was confirmed in a mouse model with Tyr319F ZAP70 mutant expression, which resulted in severe defects in calcium mobilization and thymocyte selection.

1. Chan AC, et al. 1991. Proc. Natl. Acad. Sci. 88:9166. Antigen **References:** 

- Arpaia E, *et al.* 1994. *Cell* 76:947.
  Chan AC, *et al.* 1994. *Science* 264:1599.
- 4. Negishi I, et al. 1995. Natu