ARG65562
anti-ZAP70 antibody [ZAP-03]

Package: 100 µg
Store at: -20°C

Summary

Product Description	Mouse Monoclonal antibody [ZAP-03] recognizes ZAP70
Tested Reactivity	Hu
Tested Application	FACS, ICC/IF, WB
Specificity	The clone ZAP-03 reacts with ZAP-70, a 70 kDa protein tyrosine kinase expressed in T and NK cells. ZAP-70 is a molecule susceptible to degradation. It is recommended to use freshly prepared cell lysates (protease inhibitors are essential) to avoid non-specific staining of degradation products.
Host	Mouse
Clonality	Monoclonal
Clone	ZAP-03
Isotype	IgG1
Target Name	ZAP70
Species	Human
Immunogen	Bacterially expressed fusion protein representing C-terminal part (160 amino acids) of human ZAP-70 with histidine tag
Conjugation	Un-conjugated
Alternate Names	STD; SRK; STCD; 70 kDa zeta-chain associated protein; Tyrosine-protein kinase ZAP-70; TZK; Syk-related tyrosine kinase; ZAP-70; EC 2.7.10.2

Application Instructions

Application table

Application	Dilution
FACS	2 - 5 µg/ml
ICC/IF	Assay-dependent
WB	0.5 µg/ml

Application Note

FACS: Intracellular staining.
WB: Sample preparation: Resuspend approx. 50 mil. cells in 1 ml cold lysis buffer (1% laurylmaltoside in 20 mM Tris/Cl, 100 mM NaCl pH 8.2, 50 mM NaF including Protease inhibitor Cocktail). Incubate 60 min on ice. Centrifuge to remove cell debris. Mix lysate (1:1) with reducing Laemmli SDS-PAGE sample buffer. Boil for 5 min. Application note: Reducing condition. SDS-PAGE (10% separating gel).
* The dilutions indicate recommended starting dilutions and the optimal dilutions or concentrations should be determined by the scientist.

Positive Control

FACS: Positive control: HPB-ALL cells.
WB: Positive control: HPB-ALL cells. Negative control: Ramos cells.

Properties

Form	Liquid

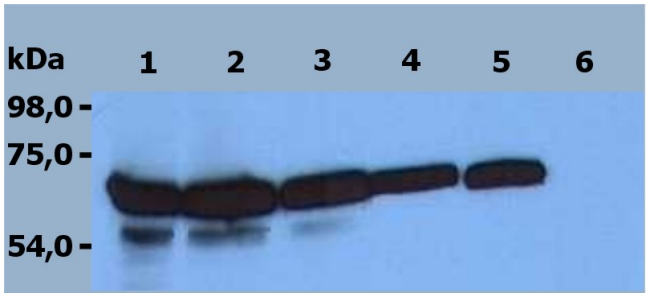
Purification	Purified from hybridoma culture supernatant by protein-A affinity chromatography.
Purity	> 95% (by SDS-PAGE)
Buffer	PBS (pH 7.4) and 15 mM Sodium azide
Preservative	15 mM Sodium azide
Concentration	1 mg/ml
Storage instruction	For continuous use, store undiluted antibody at 2-8°C for up to a week. For long-term storage, aliquot and store at -20°C or below. Storage in frost free freezers is not recommended. Avoid repeated freeze/thaw cycles. Suggest spin the vial prior to opening. The antibody solution should be gently mixed before use.
Note	For laboratory research only, not for drug, diagnostic or other use.

Bioinformation

Database links	GeneID: 7535 Human Swiss-port # P43403 Human
Gene Symbol	ZAP70
Gene Full Name	zeta-chain (TCR) associated protein kinase 70kDa
Background	The ZAP-70 (zeta-associated protein of 70 kDa) tyrosine kinase was identified as a tyrosine phosphoprotein that associates with TCR zeta subunit and undergoes tyrosine phosphorylation following TCR stimulation. ZAP-70 is a Syk family tyrosine kinase primarily expressed in T and NK cells that plays an essential role in signaling through the TCR. TCR-mediated activation of T cells is crucial to the immune response. In humans, ZAP-70 gene mutations resulting in lower ZAP-70 protein expression levels or expression of catalytically inactive ZAP-70 proteins, have been identified. ZAP-70 deficiency results in the absence of mature CD8+ T cells and the prevention of TCR-mediated activation of CD4+ T cells, and it can lead to severe combined immunodeficiency. In patients with chronic lymphocytic leukemia (B-CLL), ZAP-70 expression on B cell was shown to be correlated with disease progression and survival. ZAP-70 contains two N-terminal SH2 domains (Src homology domain 2) and a C-terminal kinase domain. During T cell activation, the binding of ZAP-70 SH2 domains to the phosphorylated zeta subunit on the activated TCR complex causes a colocalization with the Lck tyrosine kinase that phosphorylates ZAP-70 on Tyr493 in the activation loop. ZAP-70 autophosphorylates multiple tyrosines in the region between the SH2 domains and the kinase domain, including the binding sites for additional SH2-containing signaling proteins such as SLP76, LAT, Lck, PLCgamma1, Vav, Shc, Ras-GAP, and Abl. ZAP-70-mediated activation of these downstream effectors leads to the release of intracellular calcium stores, and the transcription of interleukin-2 and other genes important for an immune response.
Function	Tyrosine kinase that plays an essential role in regulation of the adaptive immune response. Regulates motility, adhesion and cytokine expression of mature T-cells, as well as thymocyte development. Contributes also to the development and activation of primary B-lymphocytes. When antigen presenting cells (APC) activate T-cell receptor (TCR), a serie of phosphorylations lead to the recruitment of ZAP70 to the doubly phosphorylated TCR component CD247/CD3Z through ITAM motif at the plasma membrane. This recruitment serves to localization to the stimulated TCR and to relieve its autoinhibited conformation. Release of ZAP70 active conformation is further stabilized by phosphorylation mediated by LCK. Subsequently, ZAP70 phosphorylates at least 2 essential adapter proteins: LAT and LCP2. In turn, a large number of signaling molecules are recruited and ultimately lead to lymphokine production, T-cell proliferation and differentiation. Furthermore, ZAP70 controls cytoskeleton modifications, adhesion and mobility of T-lymphocytes, thus ensuring correct delivery of effectors to the APC. ZAP70 is also required for TCR-CD247/CD3Z internalization and degradation through interaction with the E3 ubiquitin-protein ligase CBL and adapter proteins SLA and SLA2. Thus, ZAP70 regulates both T-cell activation switch on and switch off by modulating TCR expression at the T-cell surface. During thymocyte development, ZAP70 promotes survival and cell-cycle progression of developing thymocytes before positive selection (when cells are still CD4/CD8 double negative). Additionally, ZAP70-dependent signaling pathway may also contribute to primary B-cells formation and activation through B-cell receptor (BCR). [UniProt]
Research Area	Controls and Markers antibody; Immune System antibody; Signaling Transduction antibody; SyK / Zap70 Pathway antibody
Calculated Mw	70 kDa

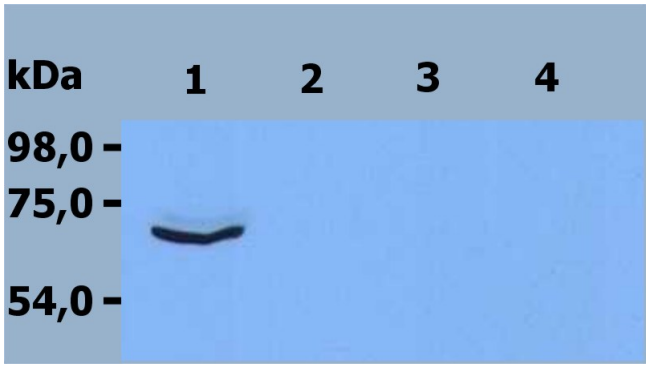
PTM	Phosphorylated on tyrosine residues upon T-cell antigen receptor (TCR) stimulation. Phosphorylation of Tyr-315 and Tyr-319 are essential for ZAP70 positive function on T-lymphocyte activation whereas Tyr-292 has a negative regulatory role. Within the C-terminal kinase domain, Tyr-492 and Tyr-493 are phosphorylated after TCR induction, Tyr-492 playing a negative regulatory role and Tyr-493 a positive. Tyr-493 is dephosphorylated by PTN22. Ubiquitinated in response to T cell activation. Deubiquitinated by OTUD7B.

Images



ARG65562 anti-ZAP70 antibody [ZAP-03] WB image

Western blot: HPB-ALL peripheral blood T cell leukemia cell lysate stained with ARG65562 anti-ZAP70 antibody [ZAP-03] at 4, 2, 1, 0.5 µg/ml (Lane 1-4), anti-ZAP-70 comparative antibody (Lane 5), and Isotype Mouse IgG1 control (Lane 6).



ARG65562 anti-ZAP70 antibody [ZAP-03] WB image

Western blot: HPB-ALL peripheral blood T cell leukemia cell lysate (Lane 1, 3), and RAMOS cell lysate (Lane 2, 4).

The blots were stained with ARG65562 anti-ZAP70 antibody [ZAP-03] at 0.5 µg/ml dilution (Lane 1, 2), or Mouse IgG1 Isotype control (Lane 3, 4).