10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

ZAP70 Antibody

Purified Mouse Monoclonal Antibody (Mab)
Catalog # AM8463b

Product Information

Application	WB, FC, IF, E
Primary Accession	P43403
Reactivity	Human
Predicted	Mouse, Rat, Bovine, Canine, Rabbit
Host	Mouse
Clonality	monoclonal
Isotype	IgG1,k
Clone Names	1475CT217.34.65
Calculated MW	69872

Additional Information

Gene ID	7535
Other Names	Tyrosine-protein kinase ZAP-70, 70 kDa zeta-chain associated protein, Syk-related tyrosine kinase, ZAP70, SRK
Target/Specificity	This ZAP70 antibody is generated from a mouse immunized with a recombinant protein.
Dilution	WB~~1:4000 FC~~1:25 IF~~1:25 E~~Use at an assay dependent concentration.
Format	Purified monoclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein G column, followed by dialysis against PBS.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Precautions	ZAP70 Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

Protein Information

Name	ZAP70
Synonyms	SRK
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. Also contributes 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 phosphorylated TCR components CD3E and CD247/CD3Z through ITAM motif at the plasma membrane (PubMed:7509083). 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).
Cellular Location	Cytoplasm. Cell membrane; Peripheral membrane protein. Note=In quiescent T-lymphocytes, it is cytoplasmic. Upon TCR activation, it is recruited at the plasma membrane by interacting with CD247/CD3Z. Colocalizes together with RHOH in the immunological synapse. RHOH is required for its proper localization to the cell membrane and cytoskeleton fractions in the thymocytes (By similarity).
Tissue Location	Expressed in T- and natural killer cells. Also present in early thymocytes and pro/pre B-cells

Background

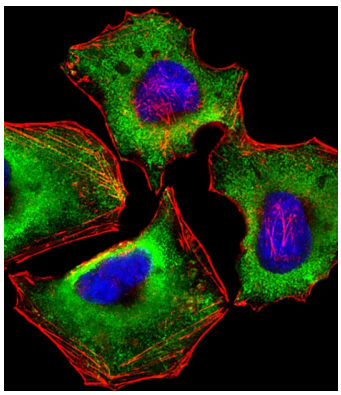
Tyrosine kinase that plays an essential role in regulation of the adaptive immune response. Regulates motility, adhesion and cytokine expression of mature T-cells, as well as thymocyte development. Contributes also to the development and activation of primary B-lymphocytes. When antigen presenting cells (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).

References

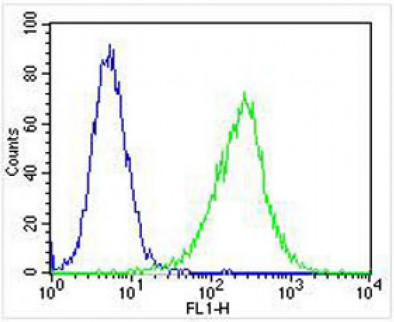
Chan A.C.,et al.Cell 71:649-662(1992).
Kuroyama H.,et al.Biochem. Biophys. Res. Commun. 315:935-941(2004).
Hillier L.W.,et al.Nature 434:724-731(2005).

Arpaia E.,et al.Cell 76:947-958(1994).
Isakov N.,et al.J. Exp. Med. 181:375-380(1995).

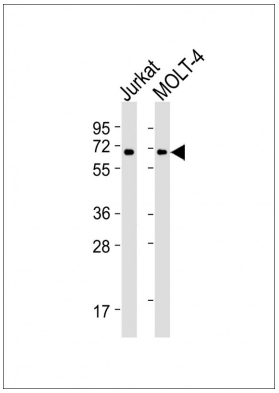
Images



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical epithelial adenocarcinoma cell line) cells labeling ZAP70 with AM8463b at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-mouse IgG (NA166821) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing cytoplasm staining on HeLa cell line. Cytoplasmic actin is detected with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red).The nuclear counter stain is DAPI (blue).



Overlay histogram showing Jurkat cells stained with AM8463b (green line). The cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with 90% methanol for 10 min. The cells were then icubated in 2% bovine serum albumin to block non-specific protein-protein interactions followed by the antibody (AM8463b, 1:25 dilution) for 60 min at 37ºC. The secondary antibody used was Goat-Anti-Mouse IgG, DyLight® 488 Conjugated Highly Cross-Adsorbed(NA168821)) at 1/400 dilution for 40 min at 37ºC. Isotype control antibody (blue line) was mouse IgG1 (1µg/1x10^6 cells) used under the same conditions. Acquisition of >10, 000 events was performed.



All lanes : Anti-ZAP70 Antibody at 1:4000 dilution Lane 1: Jurkat whole cell lysates Lane 2: MOLT-4 whole cell lysates Lysates/proteins at 20 µg per lane. Secondary Goat Anti-mouse IgG, (H+L), Peroxidase conjugated at 1/10000 dilution Predicted band size : 70 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.