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Purified Rabbit Anti-Human ZAP-70 Polyclonal Antibody

CLX228AP
Lot: R0010804

Size: 0.1 mg

Clone: Polyclonal

Isotype: Rabbit None

Specificity: C-terminal part of human ZAP-70 protein tyrosine kinase. **ZAP70 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.

Immunogen: Bacterially expressed fusion protein representing C-terminal part (160 amino acids) of human ZAP-70 with histidine tag.

Species Reactivity: Human

Application: **Western Blotting**
Recommended dilution: 1 mg/ml
Positive control: JURKAT T cell leukemia cell line
Sample preparation: Re-suspend 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 with reducing Laemmli SDS-PAGE sample buffer.
Application note: Reducing conditions.

Purity: > 95% (by SDS-PAGE)

Purification: Purified from rabbit serum by protein-A affinity chromatography

Concentration: 1 mg/ml

Storage Buffer: Phosphate buffered saline (PBS) with 15 mM sodium azide, approx. pH 7.4

For more information or to place an order please contact...

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Storage / Stability: Store at 2-8°C. Do not use after expiration date stamped on vial label. For long-term storage aliquot and store at -20°C. Avoid freeze/thaw cycles.

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. ZAP-70 is cytosolic protein migrating at 70 kDa in SDS-PAGE. It contains two N-terminal SH2 domains (Src homology domain 2) and a C-terminal kinase domain. Crystal structure of the ZAP-70 SH2 domains in complex with a TCR zeta subunit peptide was described. During T cell activation, the binding of ZAP-70 SH2 domains to the phosphorylated zeta subunit on the activated TCR complex causes a co-localization with the Lck tyrosine kinase that phosphorylates ZAP-70 on Tyr493 in the activation loop. ZAP-70 auto-phosphorylates 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 SLP-76, 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.

References:

- *Chan AC, Irving BA, Fraser JD, Weiss A: The zeta chain is associated with a tyrosine kinase and upon T-cell antigen receptor stimulation associates with ZAP-70, a 70-kDa tyrosine phosphoprotein. Proc Natl Acad Sci USA 88(20), 9166 (1991).
- *Ishaq M, DeGray G, Natarajan V: Evidence for the involvement of tyrosine kinase ZAP 70 in nuclear retinoid receptor-dependent transactivation in T lymphocytes. J Biol Chem. 2005Oct 7;280(40):34152-8.
- *Schneider H, Smith X, Liu H, Bismuth G, Rudd CE: CTLA-4 disrupts ZAP70 microcluster formation with reduced T cell/APC dwell times and calcium mobilization. Eur J Immunol. 2008 Jan;38(1):40-7.

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