SONY

Product Data Sheet

PE anti-human ZAP70 Phospho (Tyr493)

Catalog # / Size: 2580015 / 25 tests
2580020 / 100 tests

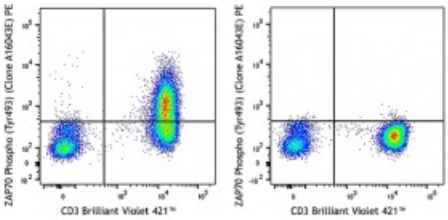
Clone: A16043E

Reactivity: Human

Preparation: The antibody was purified by affinity chromatography and conjugated with PE under optimal conditions. The solution is free of unconjugated PE and unconjugated antibody.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).

Concentration: Lot-specific



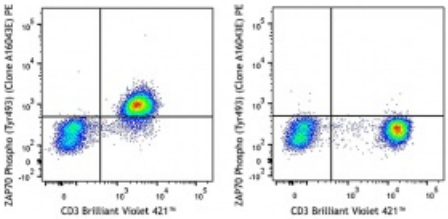
Human peripheral blood lymphocytes were treated with (left), or without (right), hydrogen peroxide for five minutes, fixed with Fixation Buffer (Cat No. 2704005), permiabilized with Intracellular Staining Permeabilization Wash Buffer (Cat No. 2705010), then intracellularly stained with CD3 Brilliant Violet 421™ and anti-ZAP70 Phospho (Tyr 493) PE (clone A16043E).

Applications:

Applications: Intracellular Flow Cytometry

Recommended Usage: Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood.

Application Notes: Positive staining is stimulation and donor dependent. Upon stimulation at high concentrations of H_20_2 some staining of B cells can be seen. Staining for ZAP70 Phospho (Tyr493) using clone A16043E can also be successfully performed using the True-Nuclear™ Transcription Buffer Set and True-Phos™ Perm Buffer.



Human peripheral blood lymphocytes were stimulated by CD3 and CD28 cross-linking (left) or unstimulated (right), fixed with Fixation Buffer (Cat. No. 2704005), permiabilized with Intracellular Staining Permeabilization Wash Buffer (Cat. No. 2705010), then intracellularly stained with CD3 Brilliant Violet 421™ and anti-ZAP70 Phospho (Tyr 493) (clone A16043E) PE. For CD3 and CD28 cross-linking, cells were incubated with anti-CD3 and anti-

CD28 on ice for 15 minutes followed by Purified anti-Mouse Ig on ice for 15 minutes, and incubated at 37°C for 2 minutes.

Application References:

Description: ZAP70 is a member of the Syk protein tyrosine kinase subfamily that is expressed exclusively in T cells and NK cells that plays an essential role in T cell receptor (TCR) signaling in combination with the Src family kinases, LCK and FYN. Upon TCR stimulation, phosphorylation of CD3 by LCK leads to the recruitment of ZAP70 to the activated receptor. Upon association of ZAP70 with the activated TCR complex, the kinase is phosphorylated on several tyrosine residues, including Tyrosine 493. Phosphorylation of Tyrosine 493, which is found within the activation loop of the protein, results in increased enzymatic activity. ZAP70 subsequently phosphorylates downstream targets, including the adaptor molecules LAT and LCP2, facilitating the recruitment and activation of other signaling molecules, ultimately leading to T cell proliferation, differentiation, and activation. ZAP70 mays also serve as a scaffold for recruiting additional factors to the activated TCR, and can function to regulate TCR-CD247/CD3Z expression at the plasma membrane. ZAP70 deficiency results in severely impaired immune function, while higher levels of ZAP70 expression are associated with certain B cell malignancies. PTN22 is required for the dephosphorylation of ZAP70 Tyrosine 493.