

Phospho-ZAP70/Syk (Tyr319, Tyr352) Monoclonal Antibody (n3kobu5), PE-Cyanine7, eBioscience™

Product Details	
Size	100 Tests
Species Reactivity	Human, Mouse
Host/Isotope	Mouse / IgG2b, kappa
Recommended Isotype Control	Mouse IgG2b kappa Isotype Control (eBMG2b), PE-Cyanine7, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	n3kobu5
Conjugate	PE-Cyanine7
Form	Liquid
Concentration	5 µl/Test
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2, with 0.2% BSA
Contains	0.09% sodium azide
Storage Conditions	4° C, store in dark, DO NOT FREEZE!

Applications	Tested	Dilution	Published
Flow Cytometry (Flow)	✓	5 µL (0.06 µg)/test	

Product Specific Information

Description: This n3kobu5 monoclonal antibody recognizes human and mouse zeta chain-associated protein of 70 kD (also known as ZAP-70) and spleen tyrosine kinase (also known as SYK) when phosphorylated on Y319 and Y352, respectively. ZAP-70 and SYK are members of the SYK protein tyrosine kinase (PTK) family that are phosphorylated and activated by Src family PTKs. ZAP-70/SYK Y319/Y352 are located in the so-called interdomain of ZAP-70/SYK (between the N-terminal dual SH2 domains and the C-terminal kinase domain).

Phosphorylation of ZAP-70 Y319 by Lck is necessary for T cell receptor (TCR)-dependent association of ZAP-70 with Lck and phospholipase C gamma and subsequent activation of calcium-dependent and Ras signaling cascades. SYK Y352 phosphorylation by Fyn/Lyn is critical for propagation of B cell receptor (BCR) signaling and for B cell development.

Specificity of this n3kobu5 clone was determined by ELISA, flow cytometry, and western blotting.

Applications Reported: This n3kobu5 antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested: This n3kobu5 antibody has been pre-diluted and tested by intracellular staining and flow cytometric analysis of sodium pervanadate-treated mouse splenocytes using the Intracellular Fixation and Permeabilization Buffer Set (Product # 88-8824-00) and protocol. Please refer to "Staining Intracellular Antigens for Flow Cytometry, Protocol A: Two step protocol for intracellular (cytoplasmic) proteins" located at thermofisher.com/flowprotocols. This may be used at 5 µL (0.06 µg) per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be

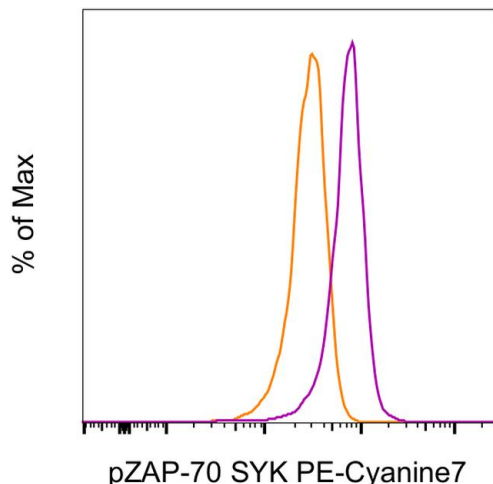
determined empirically but can range from 10^5 to 10^8 cells/test.

Light sensitivity: This tandem dye is sensitive to photo-induced oxidation. Please protect this vial and stained samples from light.

Fixation: Samples can be stored in IC Fixation Buffer (Product # 00-8222-49) (100 μ L of cell sample + 100 μ L of IC Fixation Buffer) or 1-step Fix/Lyse Solution (Product # 00-5333-57) for up to 3 days in the dark at 4°C with minimal impact on brightness and FRET efficiency/compensation. Some generalizations regarding fluorophore performance after fixation can be made, but clone specific performance should be determined empirically.

Excitation: 488-561 nm; Emission: 775 nm; Laser: Blue Laser, Green Laser, Yellow-Green Laser

Product Images For Phospho-ZAP70/Syk (Tyr319, Tyr352) Monoclonal Antibody (n3kobu5), PE-Cyanine7, eBioscience™



Phospho-ZAP70/Syk (Tyr319, Tyr352) Antibody (25-9006-42) in Flow C57BL/6 mouse splenocytes were untreated (orange histogram) or treated for 5 minutes with hydrogen peroxide-activated sodium pervanadate. Cells were then stained intracellularly, using the Intracellular Fixation and Permeabilization Buffer Set (Product # 88-8824-00) and protocol, with CD3 Monoclonal Antibody, eFluor 450 (Product # 48-0032-82) and 0.06 μ g of Phospho-ZAP70/Syk (Tyr319, Tyr352) Monoclonal Antibody, PE-Cyanine7 (purple histogram). Lymphocytes in the CD3-positive gate were used for analysis.

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