

# Phospho-ZAP70/Syk (Tyr319, Tyr352) Monoclonal Antibody (n3kobu5), PerCP-eFluor 710, eBioscience™

Product Details	
Size	100 Tests
Species Reactivity	Human, Mouse
Host/Isotope	Mouse / IgG2b, kappa
Recommended Isotype Control	Mouse IgG2b kappa Isotype Control (eBMG2b), PerCP-eFluor 710, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	n3kobu5
Conjugate	PerCP-eFluor™ 710
Form	Liquid
Concentration	5 µL/Test
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2, with 0.1% gelatin, 0.2% BSA
Contains	0.09% sodium azide
Storage Conditions	4° C, store in dark, DO NOT FREEZE!
RRID	AB_2573856

Applications	Tested	Dilution	Published
Flow Cytometry (Flow)	✓	5 µL (0.03 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-titrated and tested by intracellular staining followed by flow cytometric analysis of stimulated normal human peripheral blood cells. This can be used at 5 µL (0.03 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<sup>5</sup> to 10<sup>8</sup> cells/test.

Staining Protocol: All protocols work well for this monoclonal antibody. Use of Protocol A: Two-step protocol: intracellular (cytoplasmic) proteins allows for the greatest flexibility for detection of surface and intracellular (cytoplasmic) proteins. Use of Protocol B: One-step protocol: intracellular (nuclear) proteins is recommended for staining of transcription factors in conjunction with surface and phosphorylated intracellular (cytoplasmic) proteins. Protocol C: Two-step protocol: Fixation/Methanol allows for the greatest discrimination of phospho-specific signaling between unstimulated and stimulated samples, but with limitations on the ability to stain specific surface proteins (refer to "Clone Performance Following Fixation/Permeabilization" located in the Best Protocols Section under the Resources tab online). All Protocols can be found in the Flow Cytometry Protocols: "Staining Intracellular Antigens for Flow Cytometry Protocol" located in the Best Protocols Section under the Resources tab online.

PerCP-eFluor® 710 emits at 710 nm and is excited with the blue laser (488 nm); it can be used in place of PerCP-Cyanine5.5. We recommend using a 710/50 bandpass filter, however, the 695/40 bandpass filter is an acceptable alternative. Please make sure that your instrument is capable of detecting this fluorochrome.

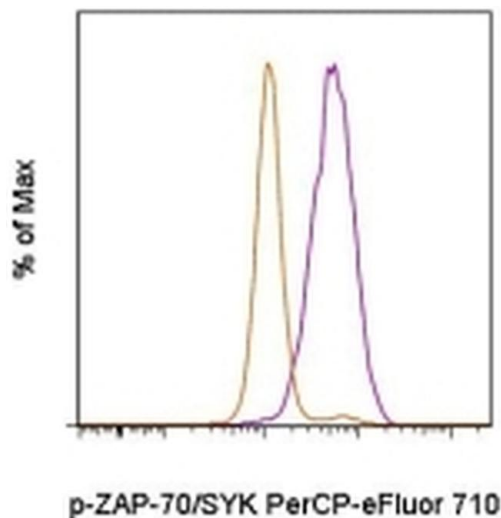
Light sensitivity: This tandem dye is sensitive to photo-induced oxidation. Please protect this vial and stained samples from light.

Fixation: Samples can be stored in IC Fixation Buffer (cat. 00-8222) (100 µL of cell sample + 100 µL of IC Fixation Buffer) or 1-step Fix/Lyse Solution (cat. 00-5333) 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 nm; Emission: 710 nm; Laser: Blue Laser.

Filtration: 0.2 µm post-manufacturing filtered.

## Product Images For Phospho-ZAP70/Syk (Tyr319, Tyr352) Monoclonal Antibody (n3kobu5), PerCP-eFluor 710, eBioscience™



**Phospho-ZAP70/Syk (Tyr319, Tyr352) Antibody (46-9006-42) in Flow**  
Intracellular staining of normal human peripheral blood cells unstimulated (orange histogram) or stimulated with hydrogen peroxide-activated sodium pervanadate for 5 minutes (purple histogram) with Anti-Human/Mouse phospho-ZAP-70/SYK (Y319 /Y352) PerCP-eFluor® 710. The Intracellular Fixation and Permeabilization Buffer Set Product # 88-8824-00) and protocol were used for staining. Lymphocytes in the CD3+ gate were used for analysis.

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