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

Product Details	
Size	100 Tests
Species Reactivity	Human, Mouse
Host/Isotype	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
Excitation/Emission Max	482/708 nm
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!
RRID	AB_2573856

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	5 µL (0.03 µg)/test	1 Publication

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 10^5 to 10^8 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.

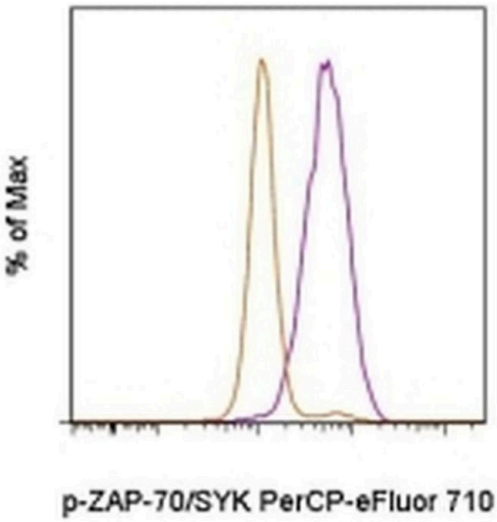
Light sensitivity: This tandem dye is sensitive to photo-induced oxidation. Please protect this vial and stained samples from light.

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 & Permeabilization Buffer Set Product # 88-8824-00) and protocol were used for staining. Lymphocytes in the CD3+ gate were used for analysis.

1 Reference

Flow Cytometry (1)

Cancers

CD19-CAR-T Cells Bearing a KIR/PD-1-Based Inhibitory CAR Eradicate $CD19^{+}HLA\text{-}C1^{-}$ Malignant B Cells While Sparing $CD19^{+}HLA\text{-}C1^{+}$ Healthy B Cells.

"Published figure using Phospho-ZAP70/Syk (Tyr319, Tyr352) monoclonal antibody (Product # 46-9006-42) in Flow Cytometry"

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2020