

Phospho-CD247 (CD3 zeta) (Tyr142) Monoclonal Antibody (3ZBR4S), APC, eBioscience™

Product Details	
Size	25 Tests
Species Reactivity	Human, Mouse
Host/Isotype	Mouse / IgG2b, kappa
Recommended Isotype Control	Mouse IgG2b kappa Isotype Control (eBMG2b), APC, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	3ZBR4S
Conjugate	APC
Immunogen	Human CD247 peptide phosphorylated on Tyr142
Form	Liquid
Concentration	5 µL/Test
Storage conditions	4° C, store in dark, DO NOT FREEZE!
RRID	AB_2744705

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	5 µL (0.25 µg)/test	-

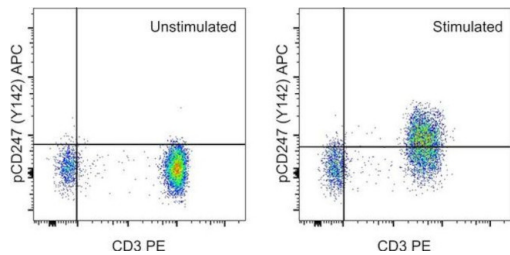
Product Specific Information

This 3ZBR4S monoclonal antibody recognizes human and mouse CD247 (CD3z) when phosphorylated on Tyrosine 142. CD3 is a multi-protein complex expressed on the surface of thymocytes and T cells, a marker of the T cell lineage, and a critical component of T cell receptor (TCR) signaling. The complex contains a gamma chain, a delta chain, and two epsilon chains, which associate with the alpha/beta or gamma/delta chains of the TCR, and the zeta chain homodimer. The CD3 zeta chain is responsible for initiating the signaling cascade following engagement of the TCR. Each CD3 zeta chain contains three immunoreceptor tyrosine-based activation motifs (ITAM) that are phosphorylated by Src kinases such as Lck. Once phosphorylated, these ITAMs form docking sites for SH2 domain-containing proteins and function to recruit ZAP-70 to the TCR where it can further amplify and propagate signaling. Loss or mutation of CD3 zeta or the ITAM motifs results in impaired T cell development, signaling, and function. CD3 zeta phosphorylation occurs early in the TCR signaling cascade. It occurs immediately downstream of Lck activation, and upstream of ZAP-70, LAT, and SLP-76.

Applications Reported: This 3ZBR4S antibody has been reported for use in flow cytometric analysis.

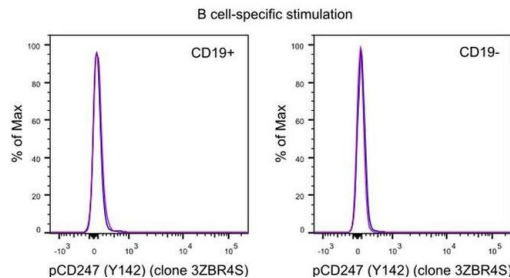
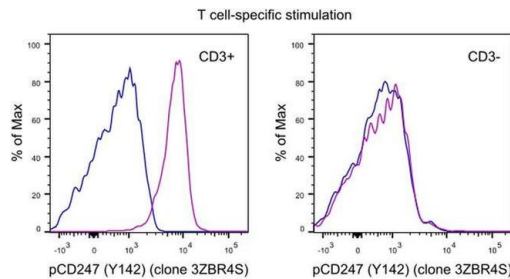
Applications Tested: This 3ZBR4S antibody has been pre-diluted and tested by flow cytometric analysis of stimulated normal human peripheral blood cells. This may be used at 5 µL (0.25 µg) per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10⁵ to 10⁸ cells/test.

Excitation: 633-647 nm; **Emission:** 660 nm; **Laser:** Red Laser



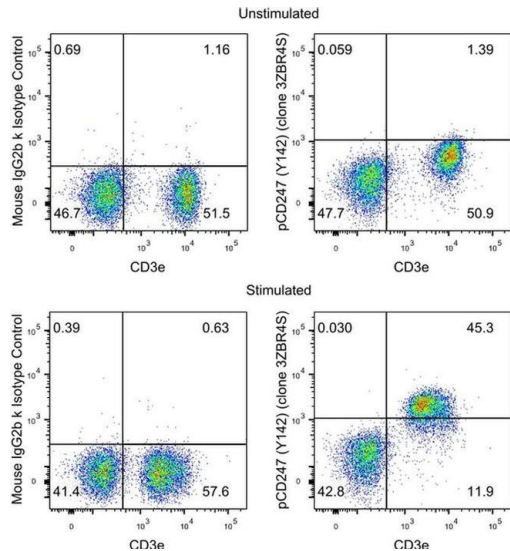
Phospho-CD247 (CD3 zeta) (Tyr142) Antibody (17-2478-41) in Flow

Normal human peripheral blood cells were unstimulated (left) or stimulated for 15 minutes with CD3 Monoclonal Antibody, Functional Grade (Product # 16-0037-85) and CD28 Monoclonal Antibody, Functional Grade (Product # 16-0288-85), followed by a 5 minute incubation with F(ab')₂-Goat anti-Mouse IgG (H+L) Secondary Antibody, Functional Grade (Product # 16-5098-85) (right). Cells were then stained intracellularly, using the Intracellular Fixation & Permeabilization Buffer Set (Product # 88-8824-00) and protocol, with CD3 Monoclonal Antibody, PE (Product # 12-0038-42) and Phospho-CD247 (Tyr142) Monoclonal Antibody, APC. Cells in the lymphocyte gate were used for analysis.



Phospho-CD247 (CD3 zeta) (Tyr142) Antibody (17-2478-41)

Intracellular staining of stimulated human peripheral blood cells. As expected based on known expression patterns, Phospho-CD247 (Tyr142) clone 3ZBR4S stains stimulated CD3+ T cells following a T cell-specific stimulation with no staining observed without stimulation, in CD3- cells, or in cells stimulated with a B cell-specific stimulation. Details: (Top Row) Human peripheral blood cells unstimulated (blue histogram) or stimulated for 15 minutes with CD3 (clone OKT3), Functional Grade and CD28 (clone 28.6), Functional Grade, followed by a 5 minute incubation with F(ab')₂-Goat anti-Mouse IgG (H+L) Secondary Antibody, Functional Grade (purple histogram) were fixed and permeabilized with the IC Fixation & Permeabilization Buffer Set and protocol, followed by intracellular staining with CD3 (clone UCHT1) and Phospho-CD247 (Tyr142) (clone 3ZBR4S). Staining of Phospho-CD247 in CD3+ cells is shown on the left, and on CD3- cells on the right. Cells in the lymphocyte gate were used for analysis. (Bottom Row) Human peripheral blood cells unstimulated (blue histogram) or stimulated for 5 minutes with F(ab')₂-Goat anti-Human IgG, IgM (H+L) Secondary Antibody, Functional Grade (purple histogram) were fixed and permeabilized with the IC Fixation & Permeabilization Buffer Set and protocol, followed by intracellular staining with CD19 (clone HIB19) and Phospho-CD247 (Tyr142) (clone 3ZBR4S). {TM}



Phospho-CD247 (CD3 zeta) (Tyr142) Antibody (17-2478-41)

Intracellular staining of stimulated mouse splenocytes. As expected based on known expression patterns, Phospho-CD247 (Tyr142) clone 3ZBR4S stains stimulated CD3+ T cells with no staining observed without stimulation, in CD3- cells, or in the isotype control. Details: Mouse splenocytes were incubated for 15 minutes with CD3e (clone 145-2C11), Functional Grade and CD28 (clone 37.51), Functional Grade, followed by a 5 minute incubation with Goat Anti-Armenian Hamster IgG Secondary Antibody (bottom row, Unstimulated in top row). Cells were fixed and permeabilized with the IC Fixation & Permeabilization Buffer Set and protocol, followed by intracellular staining with CD3e (clone eBio500A2) and Mouse IgG2b kappa Isotype Control (left) or Phospho-CD247 (Tyr142) (clone 3ZBR4S) (right). Cells in the lymphocyte gate were used for analysis. {TM}

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