

IκB alpha Monoclonal Antibody (MFRDTRK), PE, eBioscience™

Product Details	
Size	100 Tests
Species Reactivity	Human, Mouse
Published Species	Mouse
Host/Isotype	Rat / IgG2b, kappa
Recommended Isotype Control	Rat IgG2b kappa Isotype Control (eB149/10H5), PE, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	MFRDTRK
Conjugate	PE
Excitation/Emission Max	565/576 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_2572683

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	5 μL (0.125 μg)/test	1 Publication

Product Specific Information

Description: This MFRDTRK monoclonal antibody recognizes human and mouse nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha (I kappa B alpha). I kappa B alpha is one member of a family of cellular proteins that functions to inhibit classical/canonical NF-kappa B signaling by masking the nuclear localization signals (NLS) of NF-kappa B proteins and keeping them sequestered in an inactive state in the cytoplasm. Classical/canonical NF-kappa B signaling is initiated in response to myriad stimuli including engagement of T cell and B cell receptors, growth factors, and inflammatory stimuli (reactive oxygen species, TNF alpha, IL-1) and results in the activation of the I kappa B kinase (IKK) complex that includes IKK alpha, IKK beta, and NEMO. IKK phosphorylates I kappa B alpha resulting in its ubiquitination, degradation, and subsequent translocation of NF-kappa B transcription factor proteins into the nucleus.

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

Applications Tested: This MFRDTRK antibody has been pre-titrated and tested by intracellular staining followed by flow cytometric analysis of normal human peripheral blood cells. This can be used at 5 μL (0.125 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.

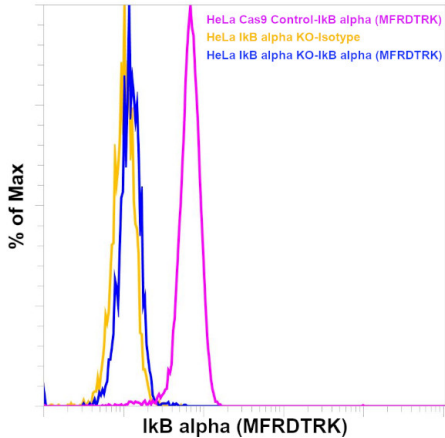
Staining Protocol: Protocol A and Protocol C are recommended for this monoclonal antibody. Use of Protocol A: Two-step protocol: intracellular (cytoplasmic) proteins allows for the greatest flexibility for detection of surface and intracellular (cytoplasmic) proteins. Protocol C: Two-step protocol: Fixation/Methanol 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.

Excitation: 488-561 nm; Emission: 578 nm; Laser: Blue Laser, Green Laser, Yellow-Green Laser.

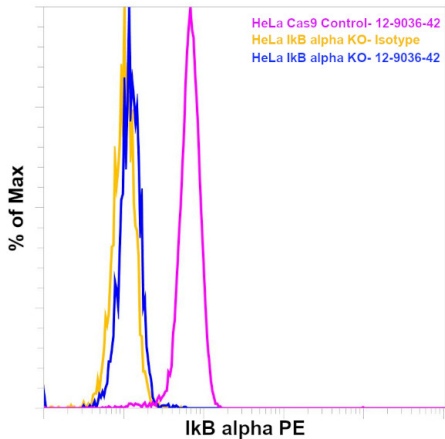
Filtration: 0.2 µm post-manufacturing filtered.

Product Images For IκB alpha Monoclonal Antibody (MFRDTRK), PE, eBioscience™



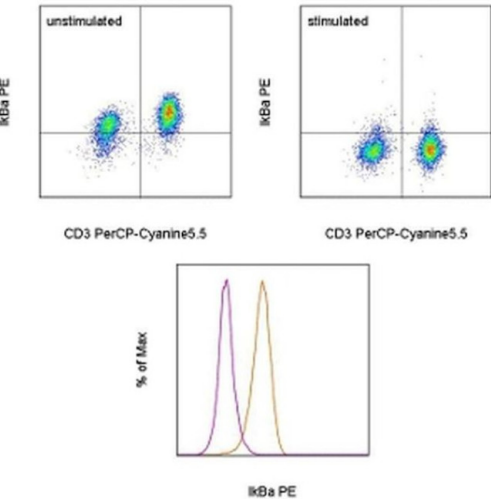
IκB alpha Antibody (12-9036-42)

Antibody clone (MFRDTRK) specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. Loss of signal was observed for target protein in MFRDTRK KO cells (blue histogram) compared to the control Cas9 cells (pink histogram) using IκB antibody (MFRDTRK). Yellow histogram represents staining with the isotype control. {KO}



IκB alpha Antibody (12-9036-42) in Flow

Knockout of IκB alpha was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042, Assay ID CRISPR993809_LV) and LentiArray Cas9 Lentivirus (Product # A32064). For Flow cytometry analysis, HeLa IκB alpha Knock out cells were stained intracellularly using the intracellular Fixation & Permeabilization Buffer Set (Product # 88-8824-00) and protocol, with 0.125 µg Rat IgG2b kappa Isotype Control (eB149/10H5), PE, eBioscience™ (Product # 12-4031-82, yellow histogram) or 0.125 µg IκB alpha Monoclonal Antibody (MFRDTRK), PE, eBioscience™ (Product # 12-9036-42, blue histogram). HeLa Cas9 control cells were also stained with 0.125 µg IκB alpha Monoclonal Antibody (MFRDTRK), PE, eBioscience™ (Product # 12-9036-42, pink histogram). Loss of signal was observed in the KO cells stained with IκB alpha antibody clone MFRDTRK but not in the control Cas9 cells. Viable cells were used for analysis, as determined by Fixable Viability Dye eFluor™780 (Product # 65-0865-18).



IκB alpha Antibody (12-9036-42) in Flow

TOP: Intracellular staining of untreated (left) or 60-minute Human TNF alpha Recombinant Protein (Product # 14-8329-81) and Calyculin A-treated (right) normal human peripheral blood cells with Anti-Human CD3 PerCP-Cyanine5-5 (Product # 45-0036-42) and Anti-Human/Mouse IκBα PE. Cells in the lymphocyte gate were used for analysis. BOTTOM: Intracellular staining of untreated (orange histogram) or 60-minute Human TNF alpha Recombinant Protein (Product # 14-8329-81) and Calyculin A-treated (purple histogram) normal human peripheral blood cells with Anti-Human/Mouse IκBα PE. CD3+ cells in the lymphocyte gate were used for analysis. In all panels, cells were stained using the using the Intracellular Fixation & Permeabilization Buffer Set (Product # 88-8824-00) and protocol.

Flow Cytometry (1)

Immunity	Year 2022
IL-17RA-signaling in Lgr5 ⁺ intestinal stem cells induces expression of transcription factor ATOH1 to promote secretory cell lineage commitment.	Species Mouse
"12-9036-42 was used in Flow cytometry/Cell sorting to suggest that the cross talk between immune cells and stem cells regulates secretory cell lineage commitment and the integrity of the mucosa."	Dilution 1:500
Authors: Lin X,Gaudino SJ,Jang KK,Bahadur T,Singh A,Banerjee A,Beaupre M,Chu T,Wong HT,Kim CK,Kempen C, Axelrad J,Huang H,Khalid S,Shah V,Eskiocak O,Parks OB,Berisha A,McAleer JP,Good M,Hoshino M,Blumberg R, Bialkowska AB,Gaffen SL,Kolls JK,Yang VW,Beyaz S,Cadwell K,Kumar P	

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