abcam

Product datasheet

Anti-Bim antibody [Y36] ab32158

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Bim antibody [Y36]
Description	Rabbit monoclonal [Y36] to Bim
Host species	Rabbit
Specificity	The antibody can recognize all isoforms of Bim. The antibody does not cross-react with other Bcl-2 family members.
Tested applications	**Suitable for:** IHC-FoFr, WB, IHC-P, Flow Cyt, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Bim aa 1-100. The exact sequence is proprietary. (Peptide available as ab179844)
Positive control	WB : Raji, Jurkat, A431, Molt-4, A20, MEF, Raw264.7 and PC-12 cell lysate; Human and mouse thymus, mouse and rat spleen tissue lysate. IHC : breast carcinoma tissue. ICC/IF : A20 and Raji cells. FC : A431 and Raji whole cell lysate. HAP1-WT cells. IP : Raji whole cell lysate.
General notes	**Mouse and Rat species are recommended based on WB results, we do not guarantee IHC-P for Mouse and Rat.** Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol, 0.05% BSA
Purity	IgG fraction
Clonality	Monoclonal
Clone number	Y36

Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab32158** in the following tested applications.

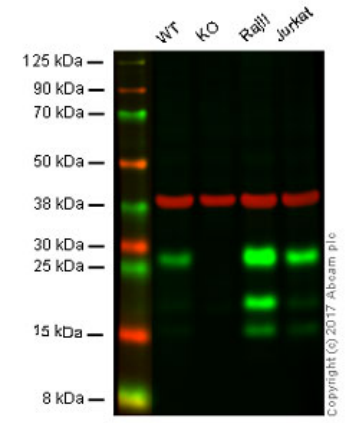
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-FoFr		1/100.
WB	★★★★★	1/500 - 1/2000. Detects a band of approximately 22 kDa (predicted molecular weight: 22 kDa).Can be blocked with Bim peptide (ab179844).
IHC-P		1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. **Mouse and Rat species are recommended based on WB results, we do not guarantee IHC-P for Mouse and Rat.**
Flow Cyt		1/50 - 1/100.
IP	★★★★★	1/40 - 1/50.
ICC/IF		1/100 - 1/250.

Target

Function	Induces apoptosis. Isoform BimL is more potent than isoform BimEL. Isoform Bim-alpha1, isoform Bim-alpha2 and isoform Bim-alpha3 induce apoptosis, although less potent than the isoforms BimEL, BimL and BimS. Isoform Bim-gamma induces apoptosis.
Tissue specificity	Isoform BimEL, isoform BimL and isoform BimS are the predominant isoforms and are ubiquitously expressed with a tissue-specific variation. Isoform Bim-gamma is most abundantly expressed in small intestine and colon, and in lower levels in spleen, prostate, testis, heart, liver and kidney.
Sequence similarities	Belongs to the Bcl-2 family.
Domain	The BH3 motif is required for Bcl-2 binding and cytotoxicity.
Cellular localization	Mitochondrion and Endomembrane system. Associated with intracytoplasmic membranes.

Images

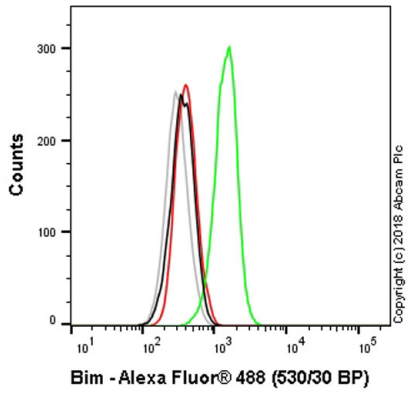


Western blot - Anti-Bim antibody [Y36] (ab32158)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: Bim knockout HAP1 whole cell lysate (20 µg)
Lane 3: Raji whole cell lysate (20 µg)
Lane 4: Jurkat whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab32158 observed at 25 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab32158 was shown to specifically react with Bim when Bim knockout samples were used. Wild-type and Bim knockout samples were subjected to SDS-PAGE. ab32158 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

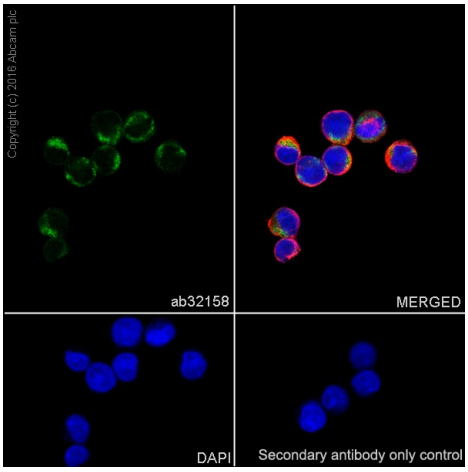


Flow Cytometry - Anti-Bim antibody [Y36] (ab32158)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-BCL2L11 knockout cells (red line) stained with ab32158. The cells were fixed 80% methanol (5 min), and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab32158, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (ab150081) at 1/2000 dilution for 30 min at 22°C.

A rabbit IgG1 isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-BCL2L11 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

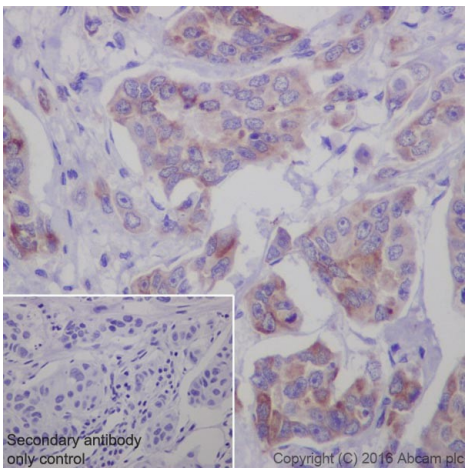


Immunocytochemistry/ Immunofluorescence - Anti-Bim antibody [Y36] (ab32158)

Immunocytochemistry/Immunofluorescence analysis of Raji (Human Burkitt's lymphoma cell line) labeling Bim with ab32158 at a dilution of 1/250. Cells were fixed with 100% methanol. Ab150077 (1/1000) was used as the secondary antibody. Cells were co-stained with ab7291, a mouse anti-tubulin antibody (1/200) using ab150120 as the secondary. Nuclei were counterstained with DAPI (blue).

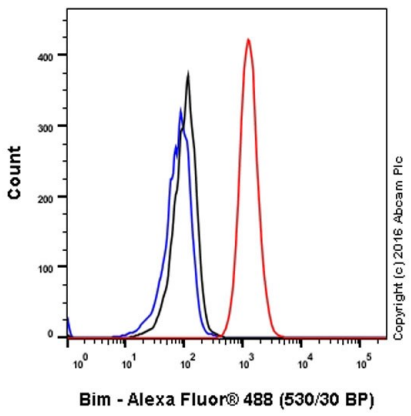
Secondary antibody only control, cells without incubation with the primary antibody was used as negative control.

Confocal image showing cytoplasmic staining on Raji cell line



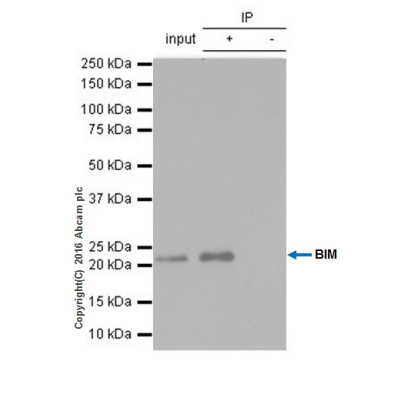
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bim antibody [Y36] (ab32158)

ab32158 staining Bim in human breast cancer tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Antigen retrieval was by heat mediated antigen retrieval using Tris/EDTA Buffer, PH9 (ab93684). Samples were incubated with primary antibody (1/100 in blocking buffer) and a Biotin-conjugated Donkey anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody. Cytoplasmic staining can be seen in the human breast cancer cells. Hematoxylin was used as a counter stain.



Flow Cytometry - Anti-Bim antibody [Y36] (ab32158)

Flow Cytometry analysis of Raji (human Burkitt's lymphoma) whole cell lysate labeling Bim with ab32158 at 1/100 (red). Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488)-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-Bim antibody [Y36] (ab32158)

Ab32158 at 1/50 immunoprecipitating Bim in Raji (human Burkitt's lymphoma) whole cell lysate.

Lane 1 (input): Raji whole cell lysate (10µg)

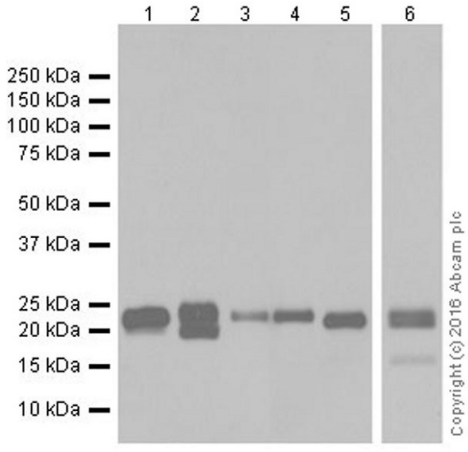
Lane 2 (+): ab32158 + Raji whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32158 in Raji whole cell lysate.

For western blotting, ab32158 (1/1000) was used as the primary antibody and ab131366 VeriBlot for IP Detection Reagent (HRP) was used for detection (1/10 000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Bim antibody [Y36] (ab32158)

All lanes : Anti-Bim antibody [Y36] (ab32158) at 1/2000 dilution

Lane 1 : Raji (human Burkitt's lymphoma) whole cell lysate
Lane 2 : A431 (human epidermoid carcinoma) whole cell lysate
Lane 3 : Molt-4 (human acute lymphoblastic leukemia) whole cell lysate
Lane 4 : Human thymus tissue lysate
Lane 5 : Mouse thymus tissue lysate
Lane 6 : A20 (mouse reticulum cell sarcoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

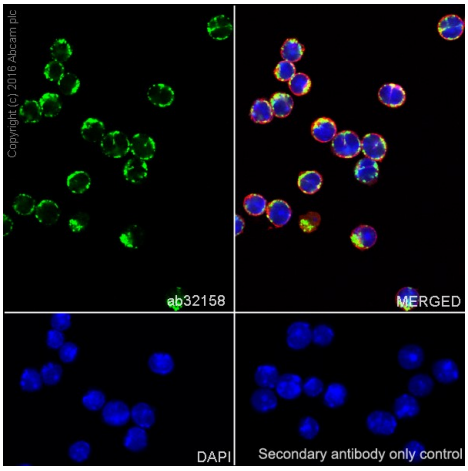
Predicted band size: 22 kDa

Observed band size : 22, 18 kDa

Exposure time : Lane 1- 5: 3 minutes; Lane 6: 2 seconds

Blocking/Diluting buffer and concentration : 5% NFDM /TBST

The observed molecular weight is consistent with the literature (PMID: 24872388)

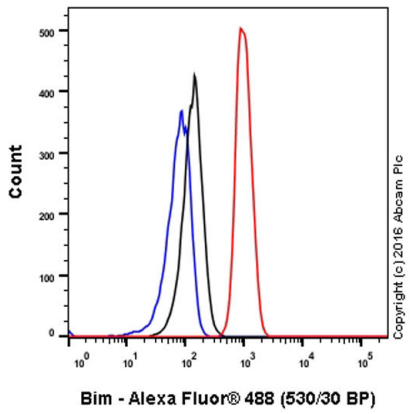


Immunocytochemistry/ Immunofluorescence - Anti-Bim antibody [Y36] (ab32158)

Immunocytochemistry/Immunofluorescence analysis of A20 (Mouse reticulum sarcoma cell line) labeling Bim with ab32158 at a dilution of 1/250. Cells were fixed with 100% methanol. Ab150077 (1/1000) was used as the secondary antibody. Cells were co-stained with ab7291, a mouse anti-tubulin antibody (1/200) using ab150120 as the secondary. Nuclei were counterstained with DAPI (blue).

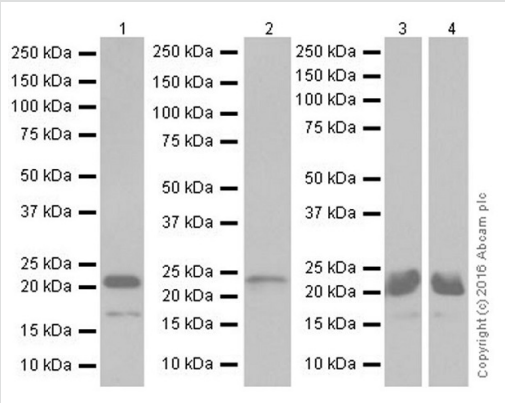
Secondary antibody only control, cells without incubation with the primary antibody was used as negative control.

Confocal image showing cytoplasmic staining on A20 cell line



Flow Cytometry - Anti-Bim antibody [Y36] (ab32158)

Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labelling Bim with ab32158 at 1/50 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-Bim antibody [Y36] (ab32158)

All lanes : Anti-Bim antibody [Y36] (ab32158) at 1/2000 dilution

Lane 1 : Mouse spleen tissue lysate
Lane 2 : Rat spleen tissue lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate
Lane 4 : Raw264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

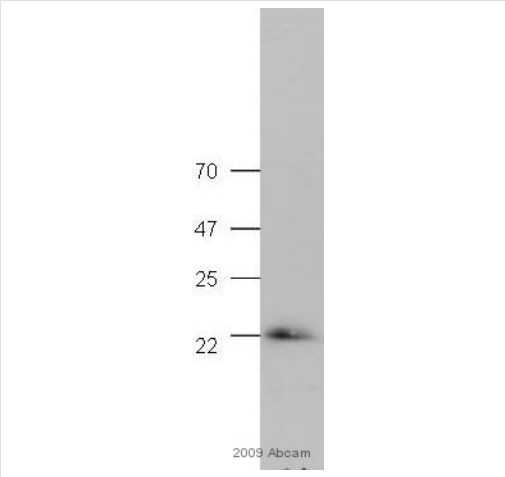
Predicted band size: 22 kDa

Observed band size : 22, 18 kDa

Exposure time : Lane 1-3: 3 minutes; Lane 4: 10 seconds

Blocking/Diluting buffer and concentration : 5% NFDM /TBST

The observed molecular weight is consistent with the literature (PMID: 24872388)



Western blot - Anti-Bim antibody [Y36] (ab32158)
This image is courtesy of an anonymous abreview.

Anti-Bim antibody [Y36] (ab32158) at 1/500 dilution + Whole cell lysates prepared from human jurkat cells at 200000 cells

Secondary
HRP conjugated Donkey polyclonal to rabbit IgG at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 22 kDa
Observed band size: 22 kDa

Exposure time: 30 seconds

Primary diluted in PBS (5% BSA + 0.1% tween20) and incubated with sample for 1 hour and 30 minutes at 20°C.

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