abcam

Product datasheet

Anti-Bcl-2 antibody [EPR17509] ab182858

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Bcl-2 antibody [EPR17509]
Description	Rabbit monoclonal [EPR17509] to Bcl-2
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment within Human Bcl-2 aa 1 to the C-terminus. The exact sequence is proprietary. Database link: P10415
Positive control	WB: Human tonsil lysate, Human thymus lysate; Jurkat, U-937, THP-1, HeLa, C2C12, WEHI-3 and NIH/3T3 whole cell lysates; Mouse brain lysate, Mouse heart lysate, Mouse kidney lysate, Mouse spleen lysate, Human fetal kidney lysate, Human fetal spleen lysate. IHC-P: Human tonsil tissue, Human endometrial cancer tissue, Mouse spleen tissue. ICC/IF: Jurkat cells. Flow Cyt: Jurkat cells.
General notes	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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17509
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab182858** in the following tested applications.

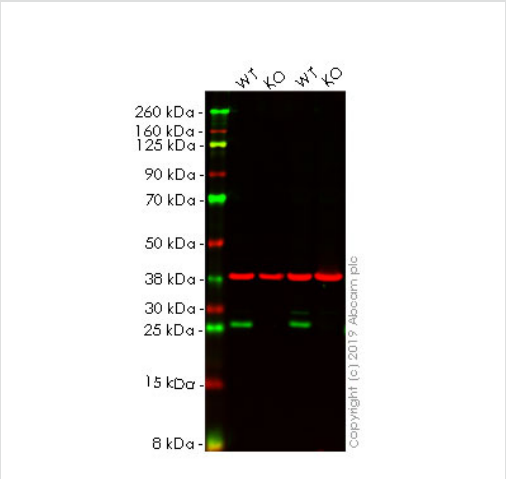
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆	1/2000. Detects a band of approximately 26 kDa (predicted molecular weight: 26 kDa).
ICC/IF	★★★★★	1/150.
Flow Cyt		1/250.
IHC-P	★★★★☆	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function	Suppresses apoptosis in a variety of cell systems including factor-dependent lymphohematopoietic and neural cells. Regulates cell death by controlling the mitochondrial membrane permeability. Appears to function in a feedback loop system with caspases. Inhibits caspase activity either by preventing the release of cytochrome c from the mitochondria and/or by binding to the apoptosis-activating factor (APAF-1). May attenuate inflammation by impairing NLRP1-inflammasome activation, hence CASP1 activation and IL1B release (PubMed:17418785).
Tissue specificity	Expressed in a variety of tissues.
Involvement in disease	A chromosomal aberration involving BCL2 has been found in chronic lymphatic leukemia. Translocation t(14;18)(q32;q21) with immunoglobulin gene regions. BCL2 mutations found in non-Hodgkin lymphomas carrying the chromosomal translocation could be attributed to the Ig somatic hypermutation mechanism resulting in nucleotide transitions.
Sequence similarities	Belongs to the Bcl-2 family.
Domain	BH1 and BH2 domains are required for the interaction with BAX and for anti-apoptotic activity. The BH4 motif is required for anti-apoptotic activity and for interaction with RAF1 and EGLN3. The loop between motifs BH4 and BH3 is required for the interaction with NLRP1.
Post-translational modifications	Phosphorylation/dephosphorylation on Ser-70 regulates anti-apoptotic activity. Growth factor-stimulated phosphorylation on Ser-70 by PKC is required for the anti-apoptosis activity and occurs during the G2/M phase of the cell cycle. In the absence of growth factors, BCL2 appears to be phosphorylated by other protein kinases such as ERKs and stress-activated kinases. Phosphorylated by MAPK8/JNK1 at Thr-69, Ser-70 and Ser-87, wich stimulates starvation-induced autophagy. Dephosphorylated by protein phosphatase 2A (PP2A). Proteolytically cleaved by caspases during apoptosis. The cleaved protein, lacking the BH4 motif, has pro-apoptotic activity, causes the release of cytochrome c into the cytosol promoting further caspase activity. Monoubiquitinated by PARK2, leading to increase its stability. Ubiquitinated by SCF(FBXO10), leading to its degradation by the proteasome.
Cellular localization	Mitochondrion outer membrane. Nucleus membrane. Endoplasmic reticulum membrane.

Images



Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

Lane 1: Hap1 wildtype cell lysate (20 µg)

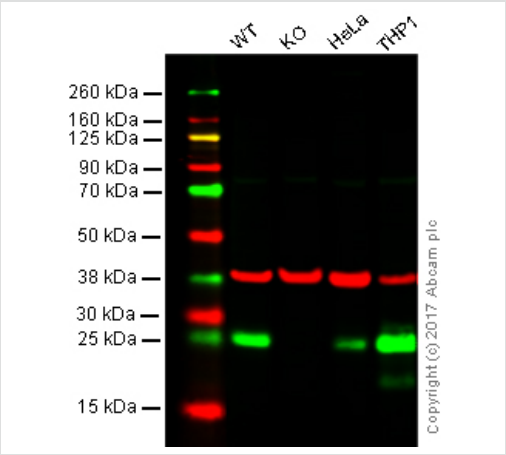
Lane 2: BCL2 Hap1 knockout cell lysate (20 µg)

Lane 3: HeLa wildtype cell lysate (20 µg)

Lane 4: BCL2 HeLa knockout cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab182858 observed at 26 kDa. Red - loading control, ab8245 observed at 37 kDa.

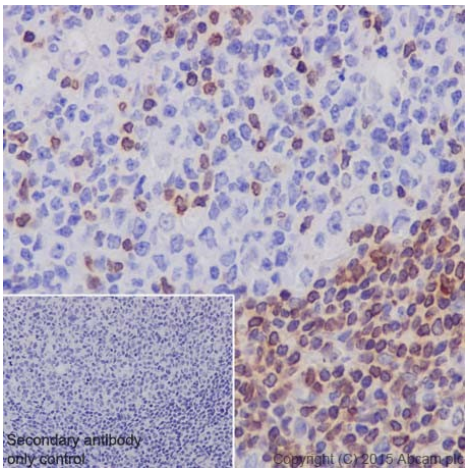
ab182858 was shown to react with Bcl-2 in HeLa wildtype. Loss of signal was observed when knockout sample ab263752 was used. Wild-type and Bcl-2 knockout samples were subjected to SDS-PAGE. ab182858 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 2000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: BCL2 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: THP-1 whole cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab182858 observed at 26 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab182858 was shown to specifically react with BCL2 when BCL2 knockout samples were used. Wild-type and BCL2 knockout samples were subjected to SDS-PAGE. Ab182858 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [EPR17509] (ab182858)

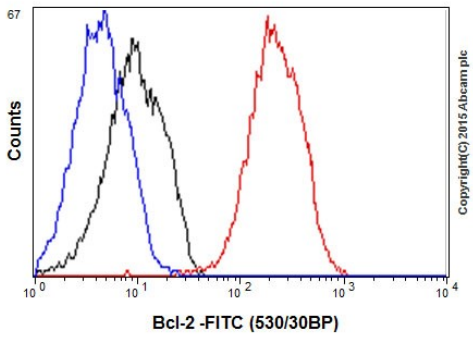
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Bcl-2 with ab182858 at 1/1000 followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500.

Cytoplasm, nuclear membrane and nucleus staining on lymphocytes of Human tonsil tissue is observed.

Counter stained with Hematoxylin.

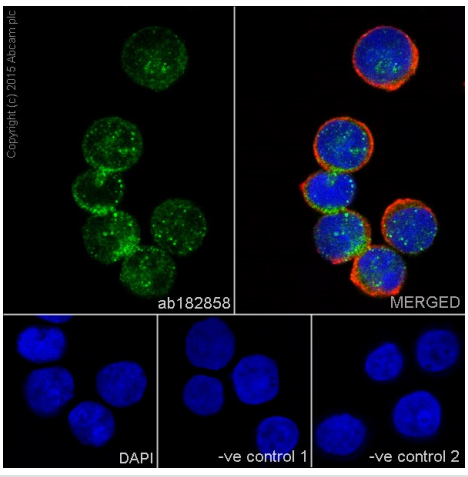
Negative control: Used PBS instead of primary antibody followed by ab97051 at 1/500.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry - Anti-Bcl-2 antibody [EPR17509] (ab182858)

Flow cytometric analysis of 4% paraformaldehyde-fixed Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling Bcl-2 with ab182858 at 1/250 (red) compared with a rabbit monoclonal IgG isotype control (ab172730) (black) and a unlabelled control (cells without incubation with primary antibody and secondary antibody (blue)). Goat anti rabbit IgG (FITC) at 1/500 was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 antibody [EPR17509] (ab182858)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling Bcl-2 with ab182858 at 1/150, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 (green).

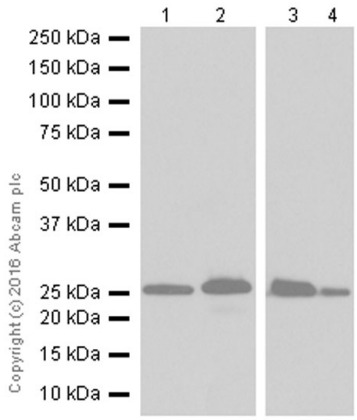
Confocal image showing cytoplasmic and weak nuclear staining on Jurkat cells line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 (red).

The negative controls are as follows:-
-ve control 1 - ab182858 at 1/150 followed by ab150120

(AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000.
-ve control 2. - ab7291 (anti-Tubulin mouse mAb) at 1/1000 followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000.



Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

All lanes : Anti-Bcl-2 antibody [EPR17509] (ab182858) at 1/10000 dilution

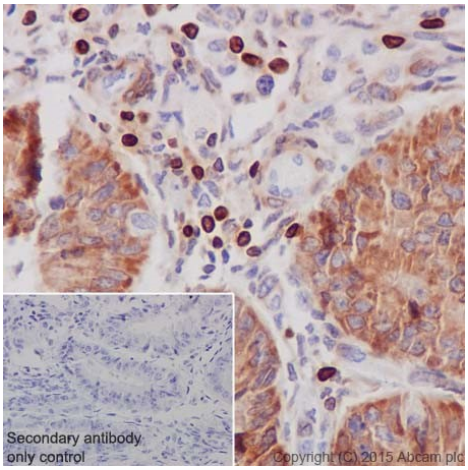
Lane 1 : NIH/3T3 (Mouse fibroblast) whole cell lysate at 20 µg
Lane 2 : WEHI-3 (Mouse myelomonocyte from leukemia) whole cell lysate at 20 µg
Lane 3 : Mouse hippocampus at 10 µg
Lane 4 : Mouse heart at 10 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) at 1/2000 dilution

Predicted band size: 26 kDa
Observed band size: 26 kDa

Exposure time: 8 seconds

Blocking/Diluting buffer 5% NFDM/TBST



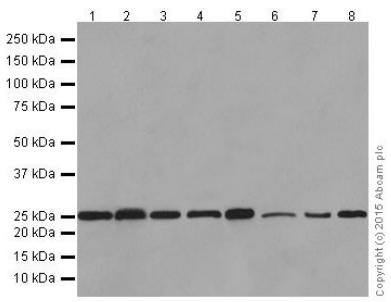
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [EPR17509] (ab182858)

Immunohistochemical analysis of paraffin-embedded Human endometrial cancer tissue labeling Bcl-2 with ab182858 at 1/1000 followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500.

Cytoplasm, nuclear membrane and nucleus staining on lymphocytes and cancer cells of Human endometrial cancer tissue is observed.

Counter stained with Hematoxylin.
Negative control: Used PBS instead of primary antibody followed by ab97051 at 1/500.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

All lanes : Anti-Bcl-2 antibody [EPR17509] (ab182858) at 1/20000 dilution

Lane 1 : Human tonsil lysate
Lane 2 : Human thymus lysate
Lane 3 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate
Lane 4 : U-937 (Human histiocytic lymphoma cells) whole cell lysate
Lane 5 : THP-1 (Human monocytic leukemia cells) whole cell lysate
Lane 6 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 7 : C2C12 (Mouse myoblast cell line) whole cell lysate
Lane 8 : WEHI-3 (Mouse leukemia cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

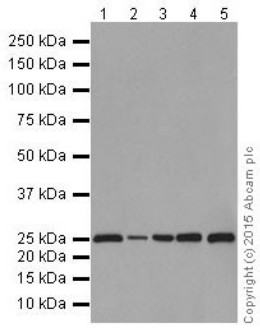
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 26 kDa
Observed band size: 26 kDa

Exposure time: 1 minute

Blocking and diluting buffer was 5% NFDM /TBST.



Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

All lanes : Anti-Bcl-2 antibody [EPR17509] (ab182858) at 1/2000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Mouse kidney lysate
Lane 4 : Mouse spleen lysate
Lane 5 : NIH/3T3 (Mouse embryo fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

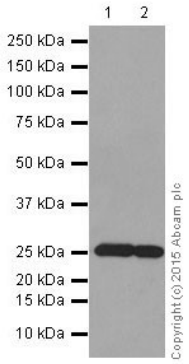
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 26 kDa
Observed band size: 26 kDa

Exposure time: 3 minutes

Blocking and diluting buffer was 5% NFDM /TBST.



Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

All lanes : Anti-Bcl-2 antibody [EPR17509] (ab182858) at 1/2000 dilution

Lane 1 : Human fetal kidney lysate
Lane 2 : Human fetal spleen lysate

Lysates/proteins at 10 µg per lane.

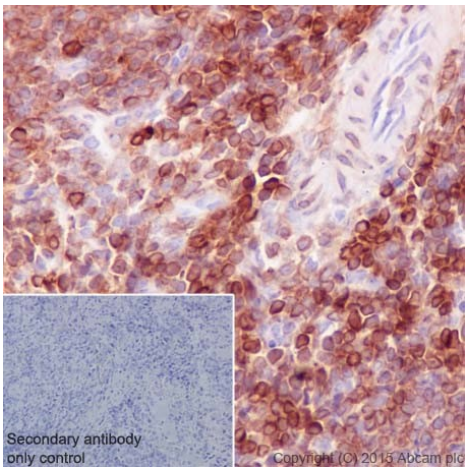
Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 26 kDa
Observed band size: 26 kDa

Exposure time: 3 minutes

Blocking and diluting buffer was 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [EPR17509] (ab182858)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling Bcl-2 with ab182858 at 1/1000 followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500.

Cytoplasm, nuclear membrane and nucleus staining on lymphocytes of Mouse spleen tissue is observed.

Counter stained with Hematoxylin.
Negative control: Used PBS instead of primary antibody followed by ab97051 at 1/500.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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