abcam

Product datasheet

Anti-Bcl-2 antibody [E17] ab32124

KO VALIDATED RabMAb

10 Abreviews 41 References 14 Images

Overview

Product name	Anti-Bcl-2 antibody [E17]
Description	Rabbit monoclonal [E17] to Bcl-2
Specificity	The antibody recognises Bcl-2. It does not cross-react with other Bcl-2 family members.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human Bcl-2 aa 50-150. Database link: P10415
Positive control	WB: MCF-7, A431, Jurkat, HeLa and SH-SY5Y cell lysates. IHC-P: human B-cell lymphona, lung adenocarcinoma and breast carcinoma tissues. ICC/IF: MCF-7 cells. Flow Cyt: Jurkat cells. IP: Jurkat cell lysate.
General notes	A trial size is available for this product. Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5, 675, 063 and/or 7, 429, 487. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** 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. Avoid freeze / thaw cycle.
Dissociation constant (K_D)	$K_D = 3.00 \times 10^{-11}$ M

Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E17
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab32124** in the following tested applications.

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

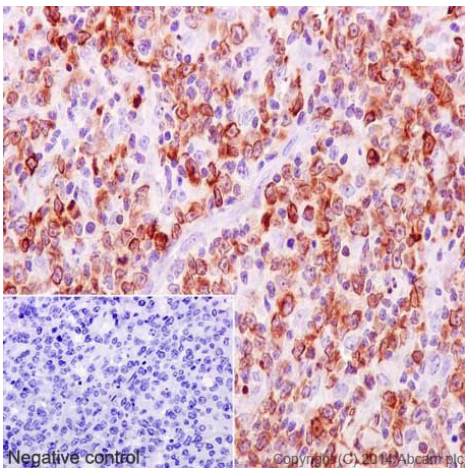
Application	Abreviews	Notes
WB	★★☆☆☆	1/1000. Detects a band of approximately 26 kDa (predicted molecular weight: 26 kDa). We recommend using ab182858 for murine samples.
IHC-P	★★★★★	1/250 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See protocols (link: http://www.abcam.com/protocols/ihc-antigen-retrieval-protocol). We do not recommend rat and mouse samples with IHC.
ICC/IF		1/100.
Flow Cyt	★☆☆☆☆	1/200 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP	★★★★★	1/50.

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.

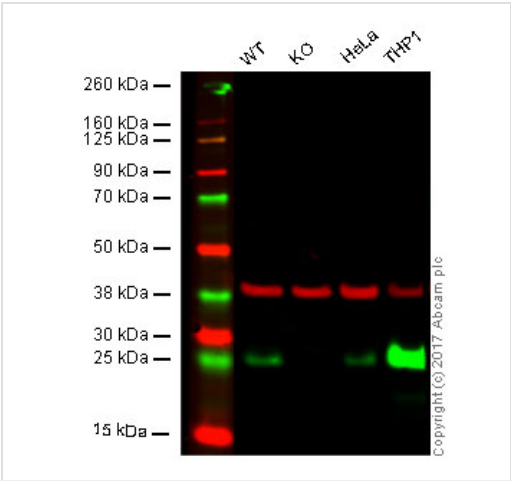
	The loop between motifs BH4 and BH3 is required for the interaction with NLRP1.
Post-translational modifications	Phosphorylation/dephosphorylation on Ser-70 regulates anti-apoptotic activity. Growth factor-stimulated phosphorylation on Ser-70 by PKC is required for the anti-apoptosis activity and occurs 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.

Anti-Bcl-2 antibody [E17] images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] (ab32124)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human B-cell lymphoma tissue labelling Bcl-2 with purified ab32124 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-Bcl-2 antibody [E17] (ab32124)

Predicted band size : 26 kDa

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

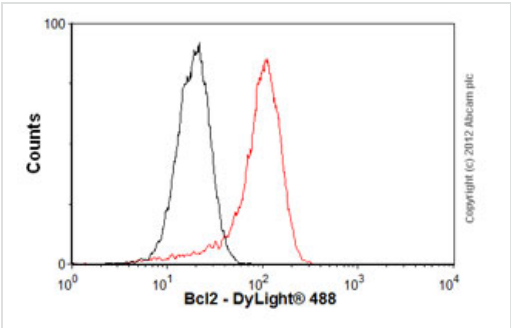
Lane 2: BCL2 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: THP-1 whole cell lysate (20 µg)

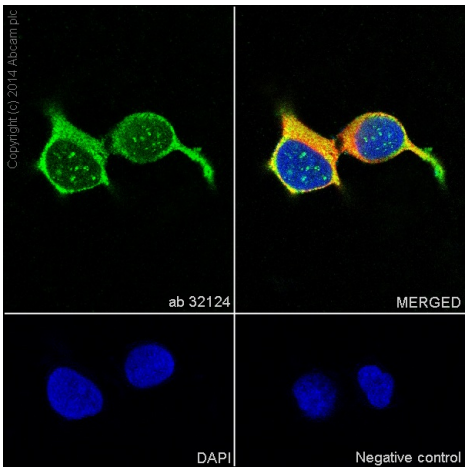
Lanes 1 - 4: Merged signal (red and green). Green - ab32124 observed at 26 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab32124 was shown to specifically react with BCL2 when BCL2 knockout samples were used. Wild-type and BCL2 knockout samples were subjected to SDS-PAGE. Ab32124 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1000 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. 3% milk used as blocking agent.



Flow Cytometry - Anti-Bcl-2 antibody [E17] (ab32124)

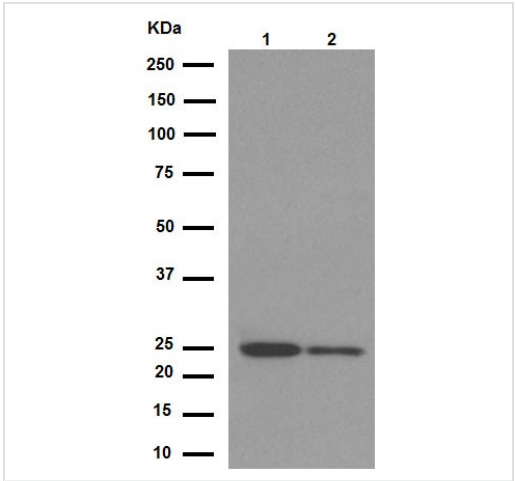
Overlay histogram showing Jurkat cells stained with unpurified ab32124 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32124, 1/100 dilution) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 antibody [E17] (ab32124)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling Bcl-2 with unpurified ab32124 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. The cells were co-stained with ab7291, a mouse anti-tubulin antibody (1/500) using ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) as the secondary. Nuclei counterstained with DAPI (blue).

Control: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).



Western blot - Anti-Bcl-2 antibody [E17] (ab32124)

All lanes : Anti-Bcl-2 antibody [E17] (ab32124) at 1/1000 dilution (puriifed)

Lane 1 : MCF-7 cell lysate
Lane 2 : A431 cell lysate

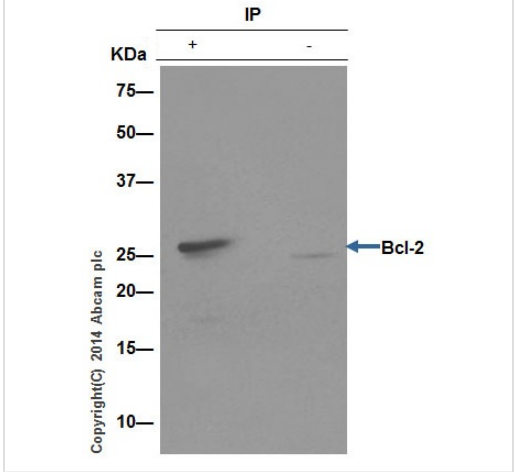
Lysates/proteins at 20 µg per lane.

Secondary
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

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

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

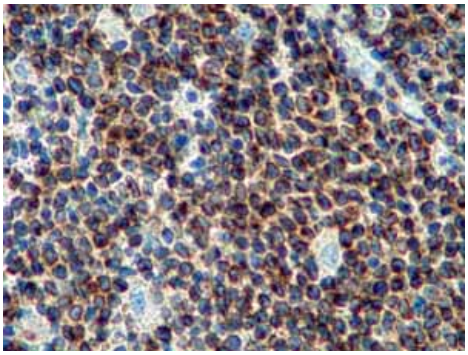


Immunoprecipitation - Anti-Bcl-2 antibody [E17] (ab32124)

ab32124 (purified) at 1/30 immunoprecipitating Bcl-2 in Jurkat cell lysate (Lane 1). Lane 2 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

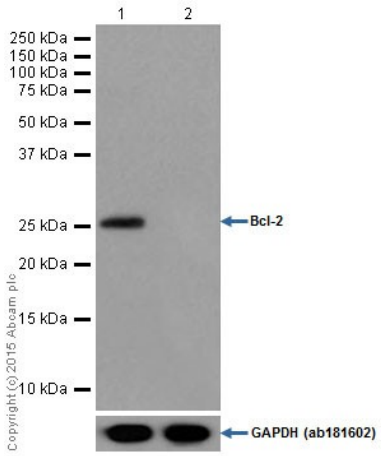
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] (ab32124)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human B cell lymphoma tissue labelling Bcl-2 with unpurified ab32124.



Western blot - Anti-Bcl-2 antibody [E17] (ab32124)

All lanes : Anti-Bcl-2 antibody [E17] (ab32124) at 1/200 dilution

Lane 1 : MCF-7
Lane 2 : SK-BR-3

Lysates/proteins at 10 µg per lane.

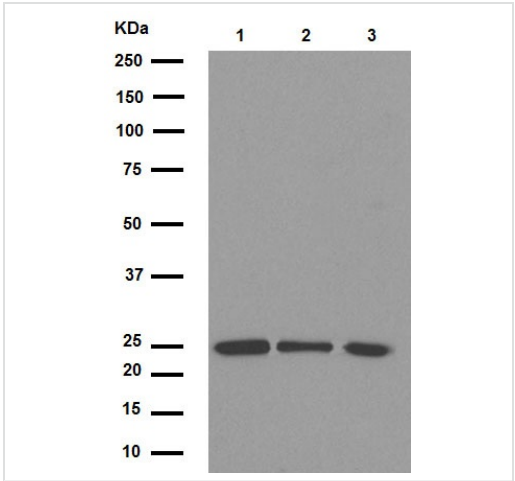
Secondary
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/50000 dilution

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

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

MCF-7 cells express Bcl-2, while SK-BR-3 cells do not express Bcl-2 (PMID: 18430249)



Western blot - Anti-Bcl-2 antibody [E17] (ab32124)

All lanes : Anti-Bcl-2 antibody [E17] (ab32124) at 1/10000 dilution (purified)

Lane 1 : Jurkat cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : SH-SY5Y cell lysate

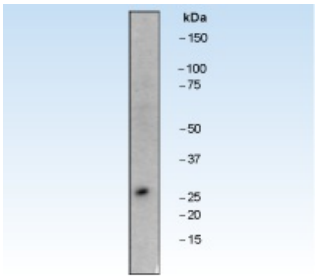
Lysates/proteins at 20 µg per lane.

Secondary
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

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

Blocking buffer and concentration: 5% NFDM/TBST.

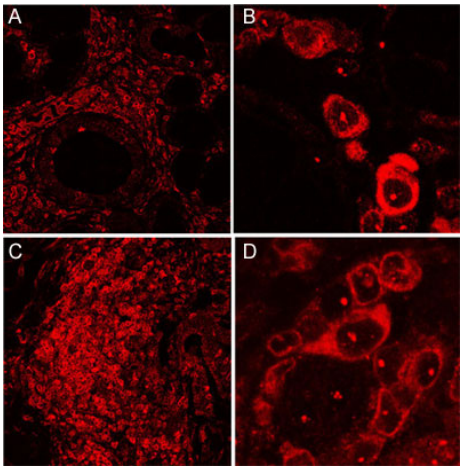
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Bcl-2 antibody [E17] (ab32124)

Anti-Bcl-2 antibody [E17] (ab32124) at 1/1000 dilution (unpurified) + Jurkat cell lysate

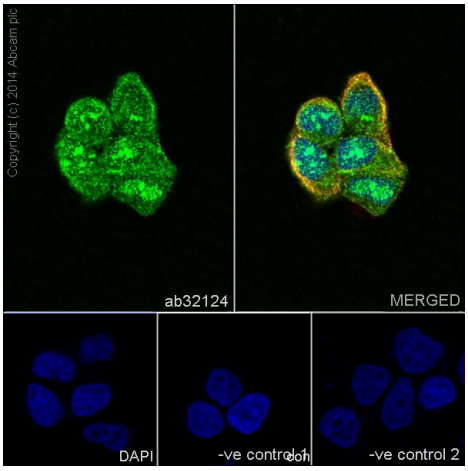
Predicted band size : 26 kDa
Observed band size : 26 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] (ab32124)

Image from Szyszko EA et al., Arthritis Res Ther. 2011 Jan 7;13(1):R2. Fig 5.; doi:10.1186/ar3220; 7 January 2011, Arthritis Research & Therapy 2011, 13:R2

Immunohistochemical analysis of Human salivary glands taken from patients with primary Sjögren's syndrome, staining Bcl-2 with unpurified ab32124.
Antigen retrieval was performed via heat mediation in a citrate buffer (pH 6). Sections were blocked using 2% BSA, 10% normal serum and permeabilized with 0.5% Triton X-100. Samples were incubated with primary antibody (1/100) for one hour at room temperature. An Alexa Fluor® 594-conjugated anti-rabbit IgG was used as the secondary antibody.
N.B. Panels B and D are higher magnifications of panels A and C, respectively.

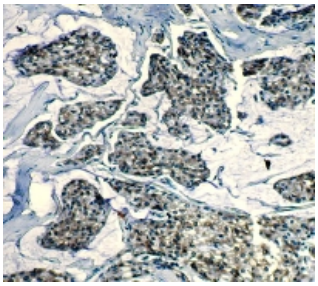


Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 antibody [E17] (ab32124)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling Bcl-2 with purified ab32124 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. The cells were co-stained with ab7291, a mouse anti-tubulin (1/500) using ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) as the secondary antibody. Nuclei counterstained with DAPI (blue).

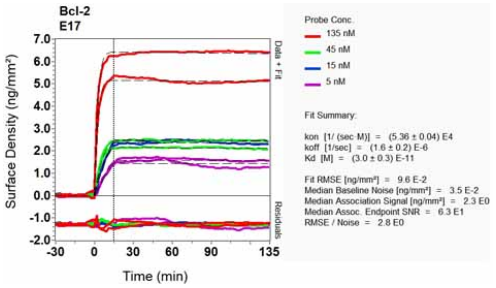
Control 1: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] (ab32124)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling Bcl-2 with unpurified ab32124 at 1/200 dilution.



Other - Anti-Bcl-2 [E17] antibody (ab32124)

Equilibrium disassociation constant (K_D)

Click here to learn more about K_D

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