abcam

Product datasheet

Anti-Bcl-2 antibody [E17] - BSA and Azide free ab185002

KO VALIDATED Recombinant RabMAb

93 References 13 Images

Overview

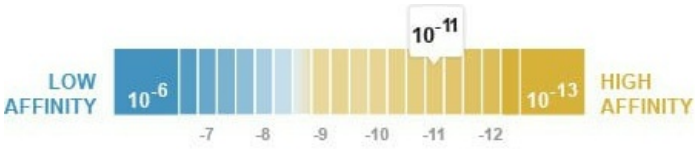
Product name	Anti-Bcl-2 antibody [E17] - BSA and Azide free
Description	Rabbit monoclonal [E17] to Bcl-2 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody recognises Bcl-2. It does not cross-react with other Bcl-2 family members. Bcl-2 has two isoforms, one is around 26kDa and the other is around 20kDa (PMID: 26009263, PMID: 10400666, PMID: 32377726).
Tested applications	**Suitable for:** WB, IP, IHC-P **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, THP-1 and MCF-7 cell lysates. IHC-P: Human DLBCL U2932, B-cell lymphoma, breast carcinoma and salivary gland tissue, and UM xenografts. IP: Jurkat cell lysate.
General notes	ab185002 is the carrier-free version of **ab32124**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production

For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 3.00 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E17
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab185002 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		Use at an assay dependent concentration. Predicted molecular weight: 26 kDa. Please check the parent abID, **ab32124**, for a recommended dilution.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

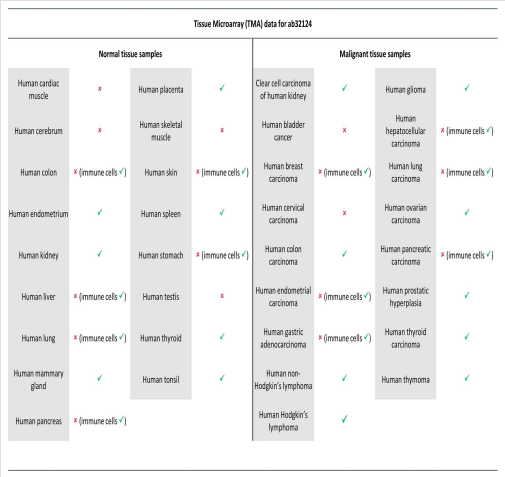
Application notes Is unsuitable for Flow Cyt or ICC/IF.

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.

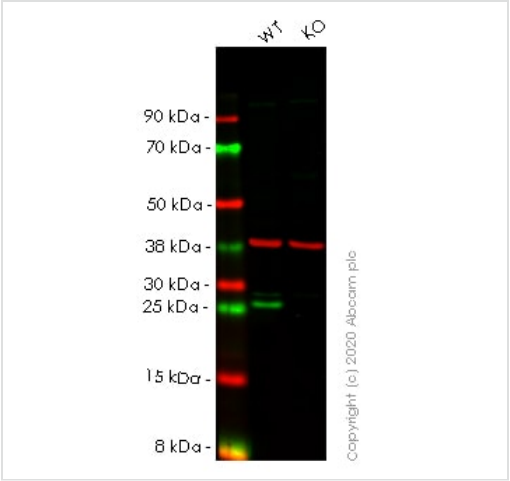
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

This data was developed using the same antibody clone in a different buffer formulation (**ab32124**).

Tissue Microarrays for Anti-Bcl2 antibody [E17] using **ab32124** in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pretreated with heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The section was incubated with **ab32124** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Western blot - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

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

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : BCL2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

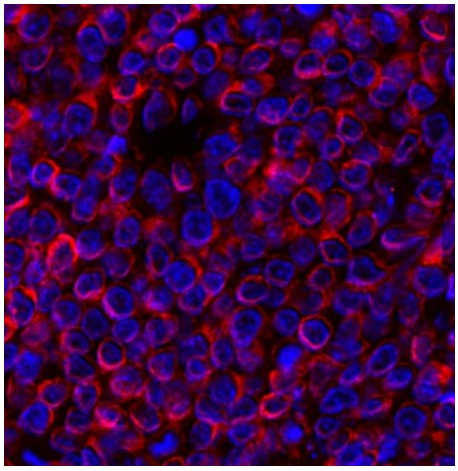
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab32124**).

Lanes 1- 2: Merged signal (red and green). Green - **ab32124** observed at 26 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32124 was shown to react with Bcl-2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255364** (knockout cell lysate **ab263752**) was used. Wild-type HeLa and BCL2 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab32124** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



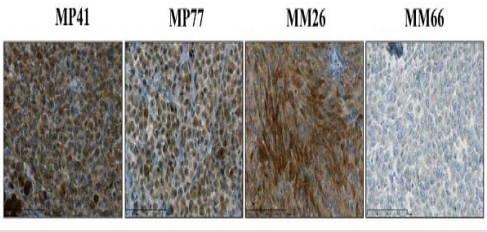
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

This image is courtesy of an anonymous Abreview.

Formaldehyde-fixed, paraffin-embedded human DLBCL U2932 cell line xenograft tissue stained for Bcl-2 using **ab32124** at 1/200 dilution in immunohistochemical analysis, followed by Goat anti-Rabbit IgG Alexa Fluor® 555.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).



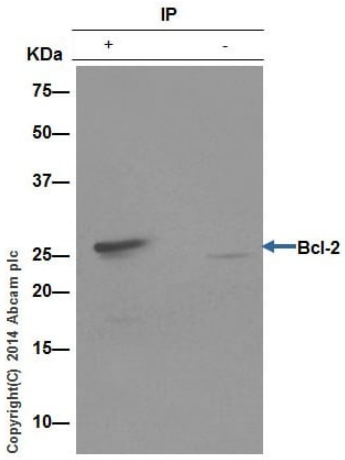
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

Image from Némati F et al. PLoS One. 2014;9(1):e80836. Fig 2.; doi: 10.1371/journal.pone.0080836.

Bcl-2 expression determined by immunohistochemical analyses of the 4 human UM xenografts (between 3 to 5 tumors have been studied per condition).

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).



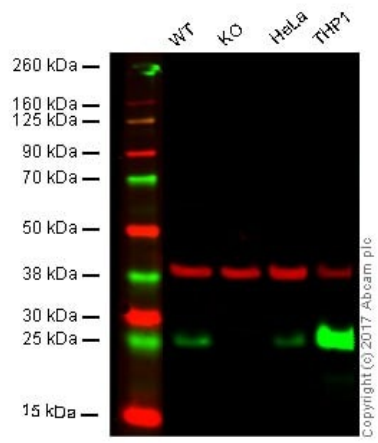
Immunoprecipitation - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

ab32124 (purified) at 1/30 immunoprecipitating Bcl-2 in Jurkat (human T cell leukemia cell line from peripheral blood) 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).



Western blot - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

All lanes : Anti-Bcl-2 antibody [E17] (**ab32124**)

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : BCL2 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : THP-1 whole cell lysate

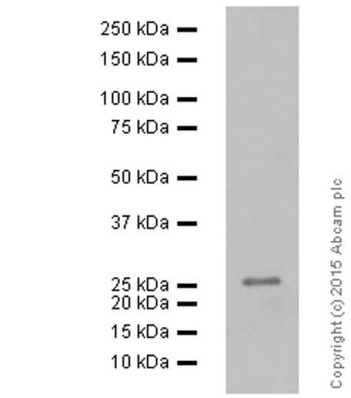
Lysates/proteins at 20 µg per lane.

Predicted band size: 26 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab32124**).

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.



Western blot - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002) + MCF-7 (human breast carcinoma) whole cell lysate at 10 µg

Secondary

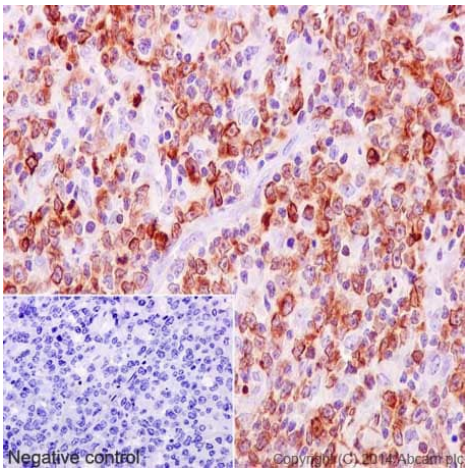
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 26 kDa

Exposure time: 3 minutes

Blocking buffer and concentration: 5% NFDM/TBST

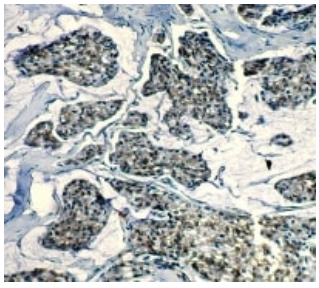
Diluting buffer and concentration: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).

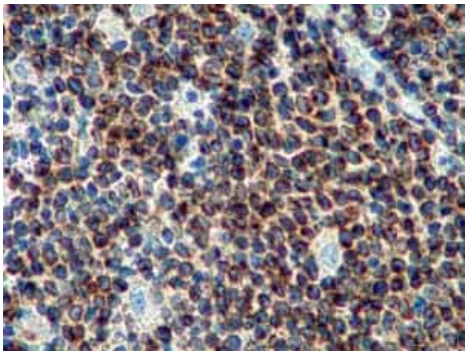


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).

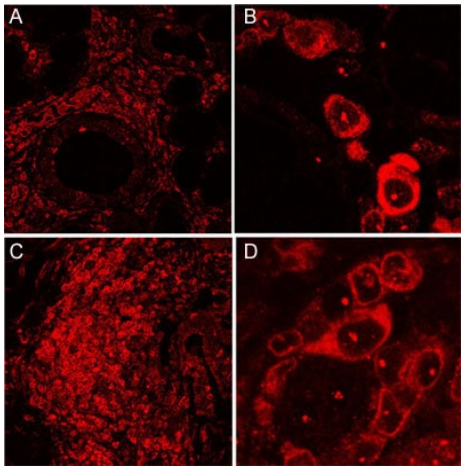


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).



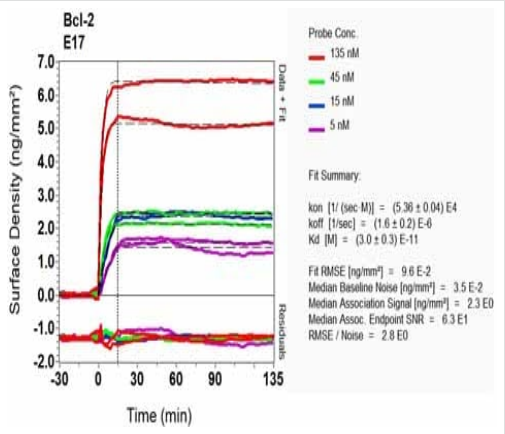
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).

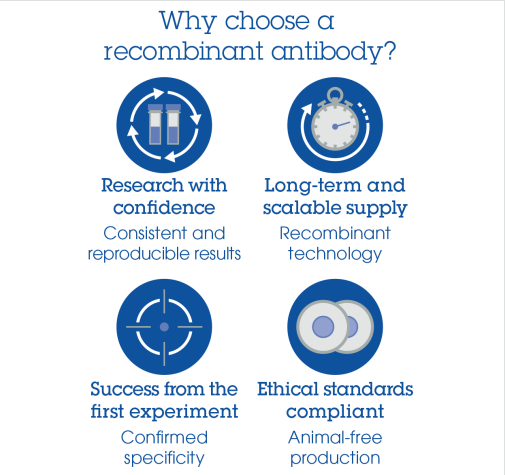


OI-RD Scanning - Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

Equilibrium disassociation constant (K_D)

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32124**).



Anti-Bcl-2 antibody [E17] - BSA and Azide free (ab185002)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors