abcam

Product datasheet

Anti-Bcl-2 antibody [E17] ab32124

KO VALIDATED Recombinant RabMAb

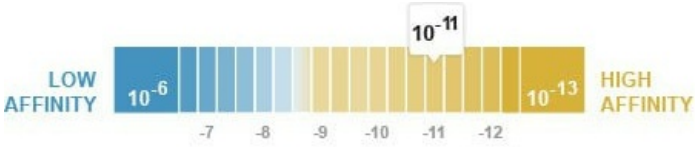
14 Abreviews 1122 References 16 Images

Overview

Product name	Anti-Bcl-2 antibody [E17]
Description	Rabbit monoclonal [E17] to Bcl-2
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, IHC-P, IP **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Bcl-2 aa 50-150. The exact sequence is proprietary. Database link: **P10415**
Positive control	WB: MCF7, A431, Jurkat, HeLa, THP-1 and SH-SY5Y cell lysates; Wild type HAP1 whole cell lysate. IHC-P: Human B-cell lymphona and breast carcinoma tissues; Human UM xenografts; Human salivary glands; Human DLBCL U2932 cell line xenograft tissue. IP: Jurkat whole cell lysate (**ab7899**).
General notes	Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). See other **anti-rabbit secondary antibodies** that can be used with this antibody. 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**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

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

The Abpromise guarantee 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	★★☆☆☆ (7)	1/1000. Detects a band of approximately 26 kDa (predicted molecular weight: 26 kDa). We recommend using **ab182858** for murine samples.
IHC-P	★★★★★ (1)	1/250 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. We do not recommend rat and mouse samples with IHC.
IP	★★★★★ (1)	1/50.

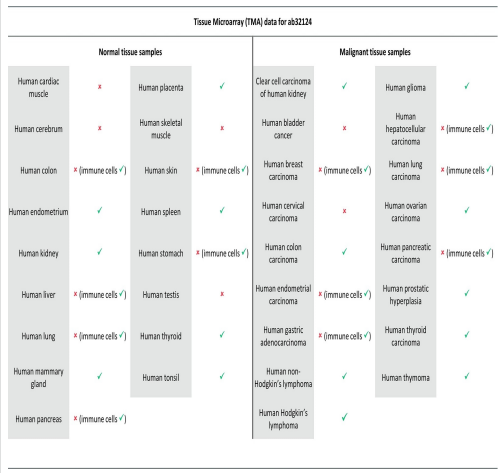
Application notes Is unsuitable for Flow Cyt or ICC/IF.

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.

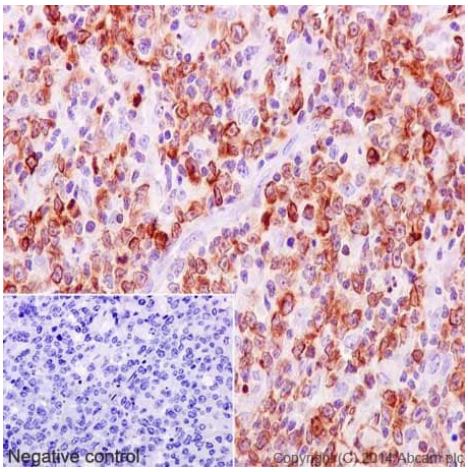
Images


Tissue Microarray (TMA) data for ab32124

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	✓	Clear cell carcinoma of human kidney	✓	Human glioma	✓
Human cerebrum	x	Human skeletal muscle	x	Human bladder cancer	x	Human hepatocellular carcinoma	x (immune cells ✓)
Human colon	x (immune cells ✓)	Human skin	x (immune cells ✓)	Human breast carcinoma	x (immune cells ✓)	Human lung carcinoma	x (immune cells ✓)
Human endometrium	✓	Human spleen	✓	Human cervical carcinoma	x	Human ovarian carcinoma	✓
Human kidney	✓	Human stomach	x (immune cells ✓)	Human colon carcinoma	✓	Human pancreatic carcinoma	x (immune cells ✓)
Human liver	x (immune cells ✓)	Human testis	x	Human endometrial carcinoma	x (immune cells ✓)	Human prostatic hyperplasia	✓
Human lung	x (immune cells ✓)	Human thyroid	✓	Human gastric adenocarcinoma	x (immune cells ✓)	Human thyroid carcinoma	✓
Human mammary gland	✓	Human tonsil	✓	Human non-Hodgkin's lymphoma	✓	Human thymoma	✓
Human pancreas	x (immune cells ✓)			Human Hodgkin's lymphoma	✓		

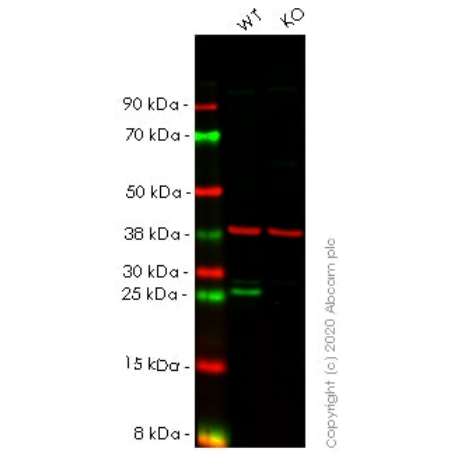
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] (ab32124)

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



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)

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.

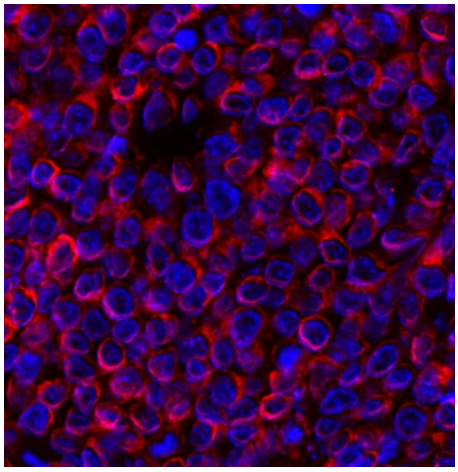
Performed under reducing conditions.

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

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 BCL2 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.

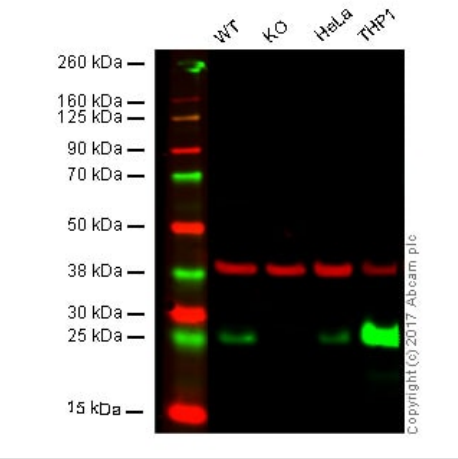
Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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

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.



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

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

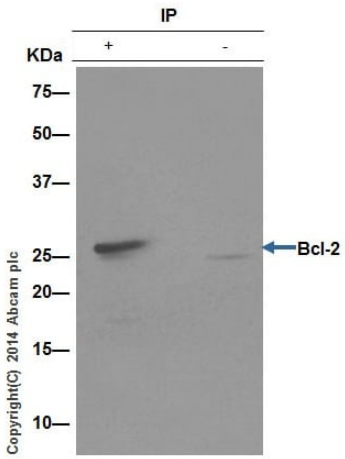
Lysates/proteins at 20 µg per lane.

Predicted band size: 26 kDa

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.

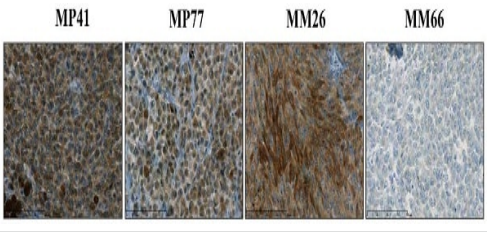


Immunoprecipitation - Anti-Bcl-2 antibody [E17] (ab32124)

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.

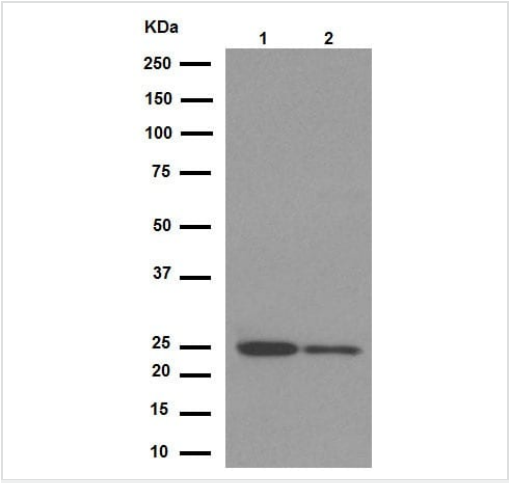
Diluting buffer and concentration: 5% NFDM /TBST.



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

Image from Némati F et al., PLoS One. 2014;9(1):e80836. Fig 2.; doi: 10.1371/journal.pone.0080836. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

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



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

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

Lane 1 : MCF7 (human breast adenocarcinoma cell line) cell lysate
Lane 2 : A431 (human epidermoid carcinoma cell line) cell lysate

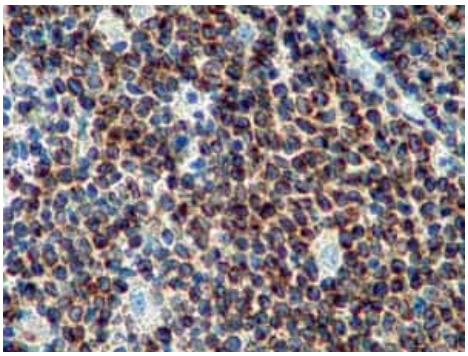
Lysates/proteins at 20 µg per lane.

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

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

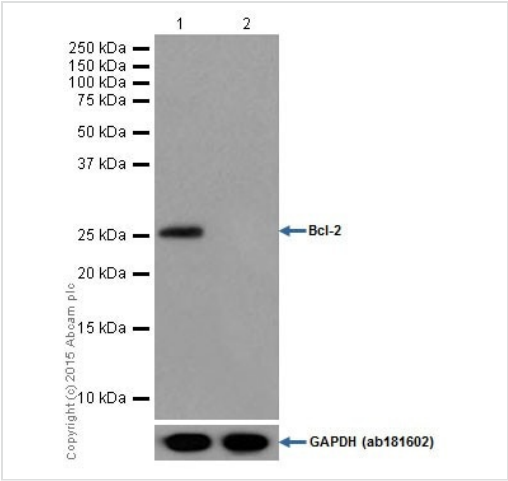
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



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. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.



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

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

Lane 1 : MCF7 (human breast adenocarcinoma cell line) cell lysate
Lane 2 : SK-BR-3 (human mammary gland adenocarcinoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

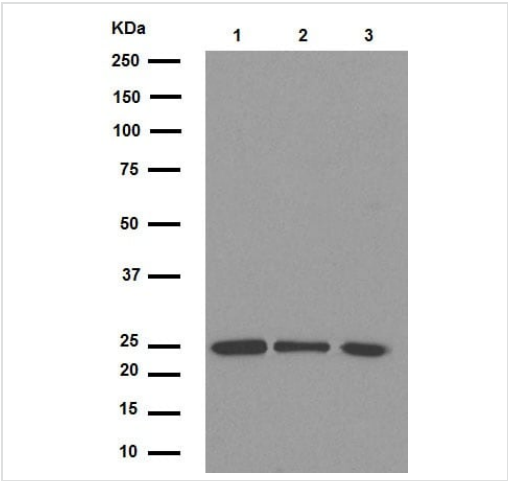
Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/50000 dilution

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 (human T cell leukemia cell line from peripheral blood) cell lysate
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 3 : SH-SY5Y (human neuroblastoma cell line from bone marrow) cell lysate

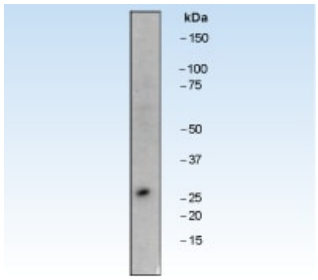
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : 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.

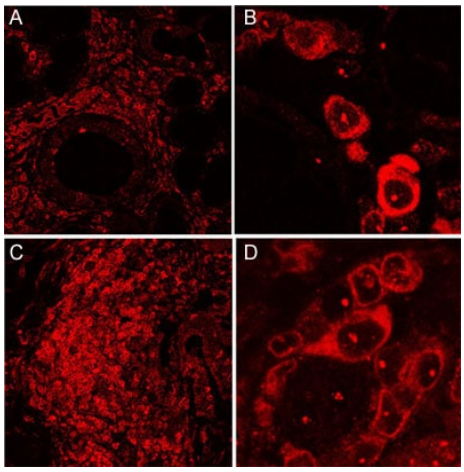
Diluting buffer and concentration: 5% NFDM /TBST.



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

Anti-Bcl-2 antibody [E17] (ab32124) at 1/1000 dilution (unpurified) + Jurkat (human T cell leukemia cell line from peripheral blood) cell lysate

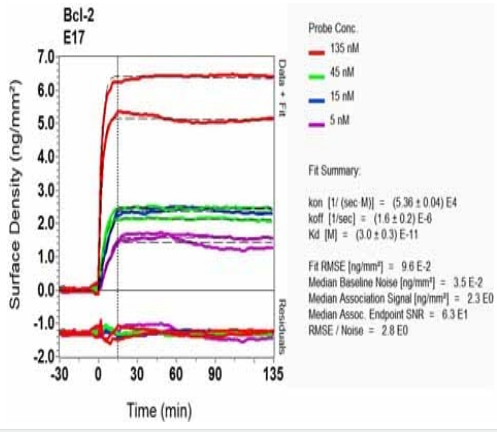
Predicted band size: 26 kDa
Observed band size: 26 kDa



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

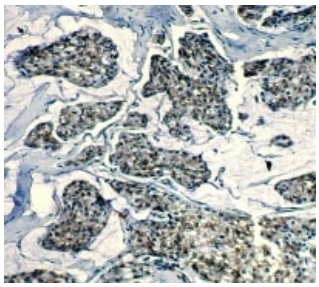
Immunohistochemical analysis of Human salivary glands taken from patients with primary Sjögren's syndrome, staining Bcl-2 with unpurified ab32124.
Antigen retrieval was performed via heat mediation in a citrate buffer (pH 6). Sections were blocked using 2% BSA, 10% normal serum and permeabilized with 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.



OI-RD Scanning - Anti-Bcl-2 antibody [E17] (ab32124)

Equilibrium disassociation constant (K_D)

Click here to learn more about K_D



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. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.

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

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