

## Product datasheet

# Anti-Bcl-2 antibody [E17] ab32124

**KO VALIDATED** Recombinant RabMAB<sup>®</sup>

★★★★☆ 11 Abreviews 137 References 16 Images

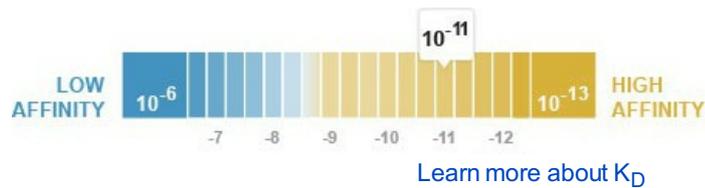
### Overview

<b>Product name</b>	Anti-Bcl-2 antibody [E17]
<b>Description</b>	Rabbit monoclonal [E17] to Bcl-2
<b>Host species</b>	Rabbit
<b>Specificity</b>	The antibody recognises Bcl-2. It does not cross-react with other Bcl-2 family members.
<b>Tested applications</b>	<b>Suitable for:</b> WB, IHC-P, ICC/IF, IP <b>Unsuitable for:</b> Flow Cyt
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Synthetic peptide within Human Bcl-2 aa 50-150. The exact sequence is proprietary. Database link: <a href="#">P10415</a>
<b>Positive control</b>	WB: MCF7, A431, Jurkat, HeLa, THP-1 and SH-SY5Y cell lysates; Wild type HAP1 whole cell lysate. IHC-P: Human B-cell lymphoma and breast carcinoma tissues; Human UM xenografts; Human salivary glands; Human DLBCL U2932 cell line xenograft tissue. ICC/IF: MCF7 and HeLa cells. Flow Cyt: Jurkat cells. IP: Jurkat cell lysate.
<b>General notes</b>	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.  Our RabMAB <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMab<sup>®</sup> patents</a> .  <b>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.</b>  This product is a <a href="#">recombinant rabbit monoclonal antibody</a> .

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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<sub>D</sub>)** K<sub>D</sub> = 3.00 x 10<sup>-11</sup> M



**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 <a href="#">ab182858</a> 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 <a href="#">IHC antigen retrieval protocols</a> . We do not recommend rat and mouse samples with IHC.
ICC/IF	★ ★ ★ ★ ★	1/100.
IP	★ ★ ★ ★ ★	1/50.

**Application notes** Is unsuitable for Flow Cyt.

## 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.

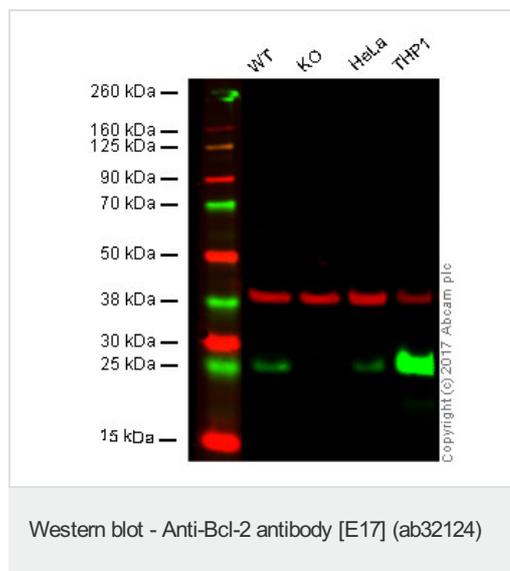
### Post-translational modifications

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, which 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



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

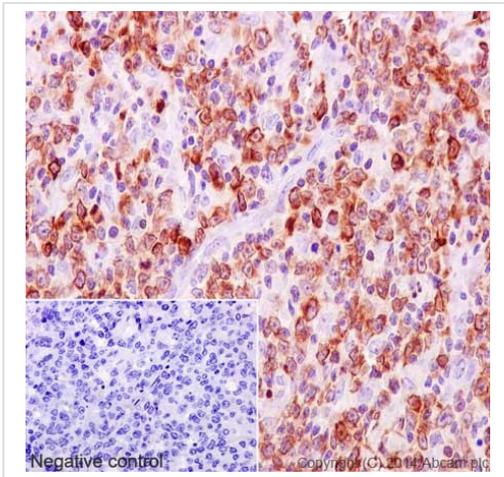
**Lane 2:** BCL2 knockout HAP1 whole cell lysate (20 µg)

**Lane 3:** HeLa whole cell lysate (20 µg)

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

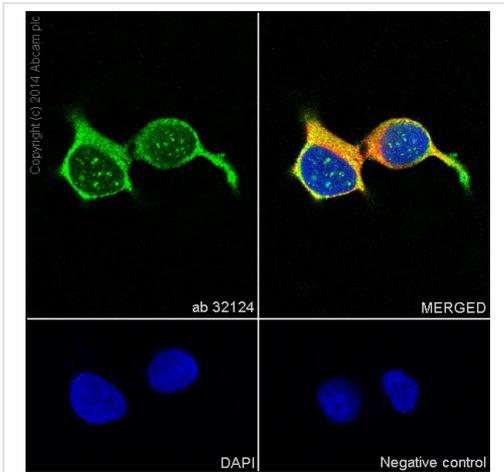
**Lanes 1 - 4:** Merged signal (red and green). Green - ab32124 observed at 26 kDa. Red - loading control, ab8245, observed at 37 kDa.

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.



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

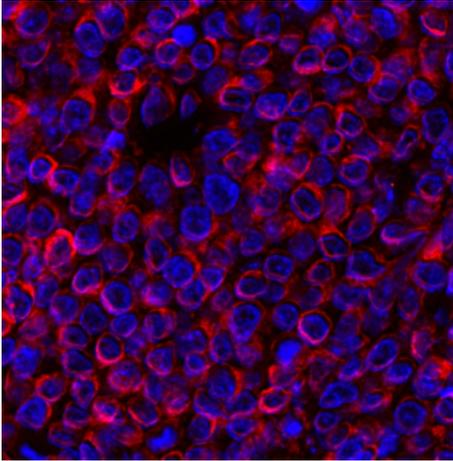
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human B-cell lymphoma tissue labelling Bcl-2 with purified ab32124 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as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



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

Immunocytochemistry/Immunofluorescence analysis of MCF7 (human breast adenocarcinoma cell line) 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<sup>®</sup> 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<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/500) as the secondary. Nuclei counterstained with DAPI (blue).

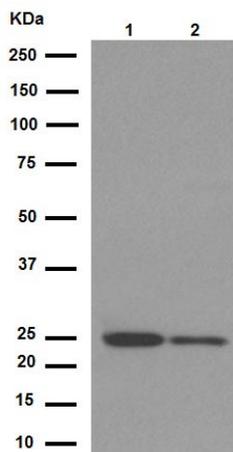
Control: primary antibody (1/100) and secondary antibody, [ab150120](#), an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/500).



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<sup>®</sup> 555.



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

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

**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

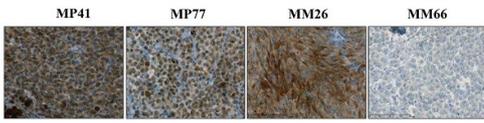
**All lanes** : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

**Predicted band size:** 26 kDa

**Observed band size:** 26 kDa

Blocking buffer and concentration: 5% NFDm/TBST.

Diluting buffer and concentration: 5% NFDm /TBST.

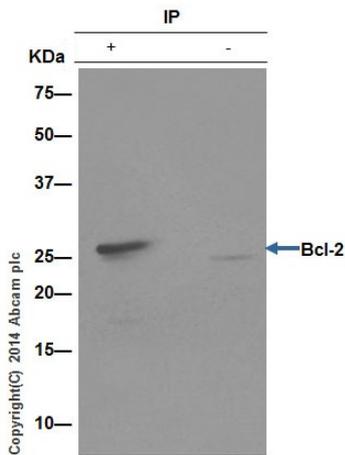


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17]

(ab32124)

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).



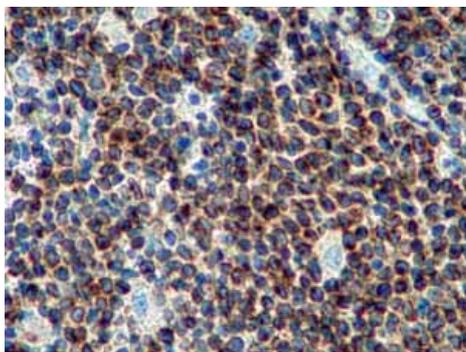
Immunoprecipitation - Anti-Bcl-2 antibody [E17]

(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.

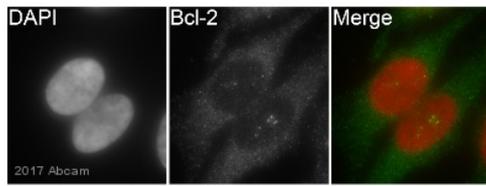
Diluting buffer and concentration: 5% NFDm /TBST.



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

(ab32124)

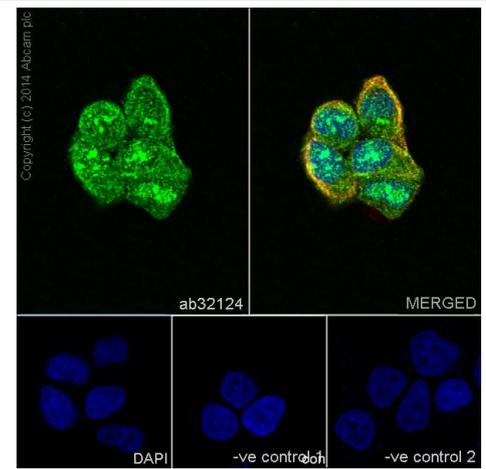
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human B cell lymphoma tissue labelling Bcl-2 with unpurified ab32124.



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

This image is courtesy of an Abreview submitted by Dr. Kirk McManus.

Methanol-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained for Bcl-2 using ab32124 at 1/200 dilution in ICC/IF, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) preadsorbed ([ab150081](#)). Counter-stained with DAPI.

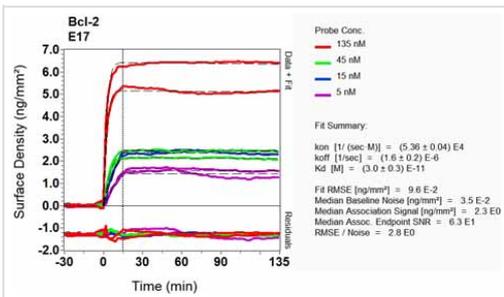


Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 antibody [E17] (ab32124)

Immunocytochemistry/Immunofluorescence analysis of MCF7 (human breast adenocarcinoma cell line) 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/500).

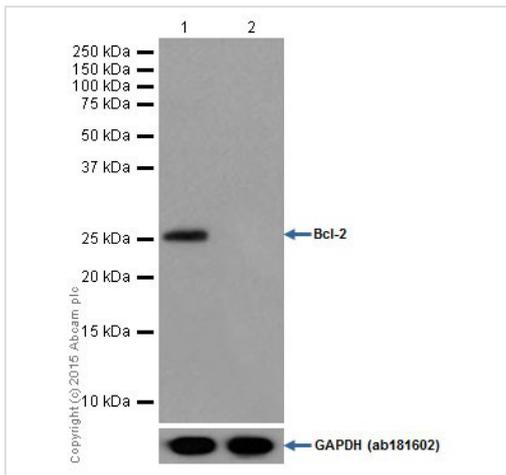
Control 2: [ab7291](#) (1/1000) and secondary antibody, [ab150077](#), an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/500).



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

Equilibrium dissociation constant ( $K_D$ )

[Click here to learn more about  \$K\_D\$](#)



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

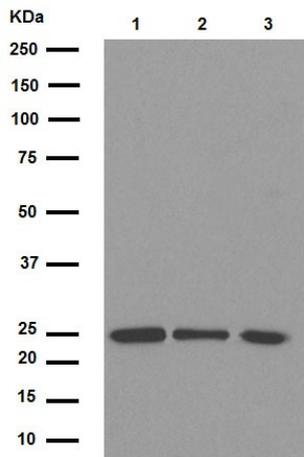
**Predicted band size:** 26 kDa

**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

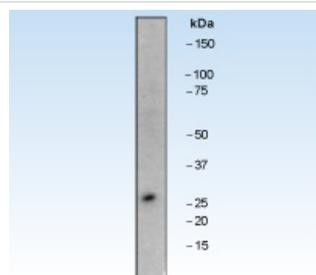
**All lanes :** Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

**Predicted band size:** 26 kDa

**Observed band size:** 26 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

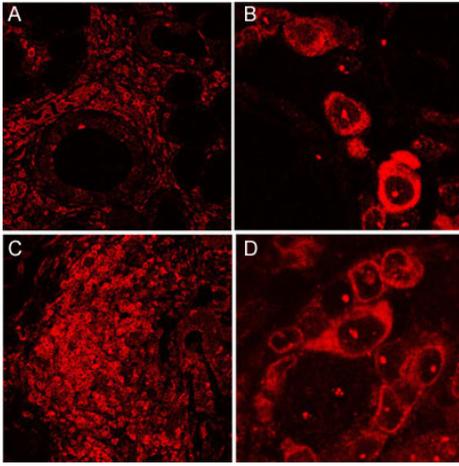


Western blot - Anti-Bcl-2 antibody [E17] (ab32124)

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



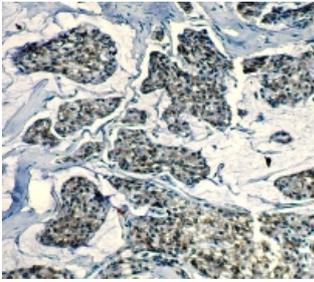
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [E17] (ab32124)

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<sup>®</sup> 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.



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.

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