**Product datasheet**

**Anti-Bcl-2 antibody [EPR17509] ab182858**

**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 and thymus lysates; Jurkat, U-937, THP-1, HeLa, C2C12, WEHI-3 and NIH/3T3 whole cell lysates; Mouse brain, heart, kidney and spleen lysates; Human fetal kidney and fetal spleen lysates; Wild-type Hap1 cell lysate. IHC-P: Human tonsil tissue, Human endometrial cancer tissue, Mouse spleen tissue. ICC/IF: Jurkat cells. Flow Cyt: Jurkat cells.

**General notes**
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**

**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.

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

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**

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

- **Lane 1**: Wild-type Hap1 cell lysate
- **Lane 2**: BCL2 knockout Hap1 cell lysate
- **Lane 3**: HeLa wildtype cell lysate
- **Lane 4**: BCL2 HeLa knockout cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size**: 26 kDa

**Observed band size**: 26 kDa

**Lanes 1-4**: 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.
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 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.

**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 µg/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.
dilution for 1 hour at room temperature before imaging.

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

**Lane 1**: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate at 20 µg
**Lane 2**: WEHI-3 (mouse leukemia cell line) 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

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.
**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.
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 cell line) 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.
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.
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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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