abcam

Product datasheet

# Anti-Bcl-2 antibody [EPR17509] ab182858

敲除验证 重组 RabMAb

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## 概述

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|---|---|
| **产品名称** | Anti-Bcl-2抗体[EPR17509] |
| **描述** | 兔单克隆抗体[EPR17509] to Bcl-2 |
| **宿主** | Rabbit |
| **经测试应用** | **适用于:** Flow Cyt (Intra), WB, IHC-P<br>**不适用于:** ICC/IF |
| **种属反应性** | **与反应:** Mouse, Human |
| **免疫原** | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| **阳性对照** | 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. Flow Cyt (intra): Jurkat cells. |
| **常规说明** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## 性能

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| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. |
| **存储溶液** | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: 59% PBS, 40% Glycerol, 0.05% BSA |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |

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| **克隆编号** | EPR17509 |
| **同种型** | IgG |

## 应用

**The Abpromise guarantee** **Abpromise™承诺保证使用ab182858于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

| 应用 | Ab评论 | 说明 |
|---|---|---|
| **Flow Cyt (Intra)** | | 1/250. |
| **WB** | ★★★★★ (5) | 1/2000. Detects a band of approximately 26 kDa (predicted molecular weight: 26 kDa). |
| **IHC-P** | ★★★★☆ (7) | 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

**应用说明** Is unsuitable for ICC/IF.

## 靶标

**功能** 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).

**组织特异性** Expressed in a variety of tissues.

**疾病相关** 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.

**序列相似性** Belongs to the Bcl-2 family.

**结构域** 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.

**翻译后修饰** 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),

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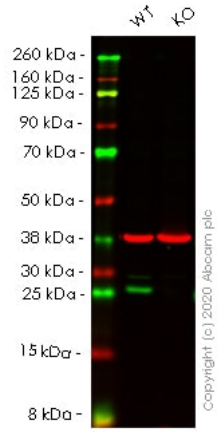
| | leading to its degradation by the proteasome. |
| **细胞定位** | Mitochondrion outer membrane. Nucleus membrane. Endoplasmic reticulum membrane. |

## 图片




Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

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

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

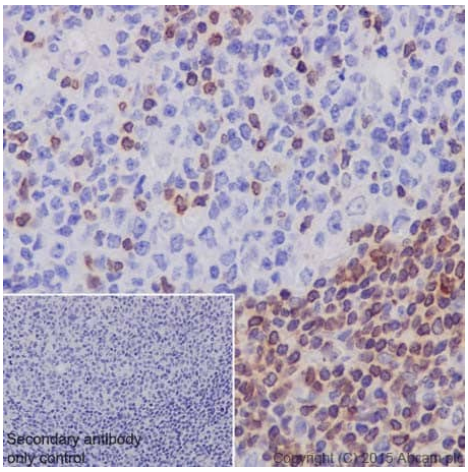
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**Lanes 1- 2:** Merged signal (red and green). Green - ab182858 observed at 26 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab182858 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. ab182858 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 2000 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 [EPR17509] (ab182858)

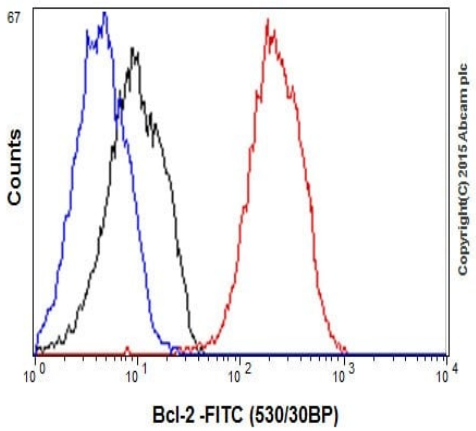
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Bcl-2 with ab182858 at 1/1000 followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500.

Cytoplasm, nuclear membrane and nucleus staining on lymphocytes of Human tonsil tissue is observed.

Counter stained with Hematoxylin.

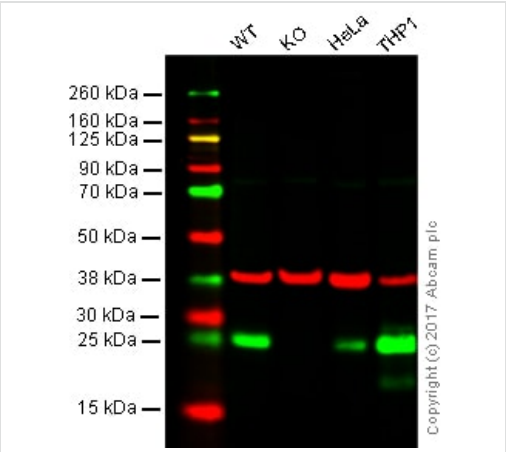
Negative control: Used PBS instead of primary antibody followed by **ab97051** at 1/500.

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




Flow Cytometry (Intracellular) - Anti-Bcl-2 antibody [EPR17509] (ab182858)

Intracellular 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.




Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

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

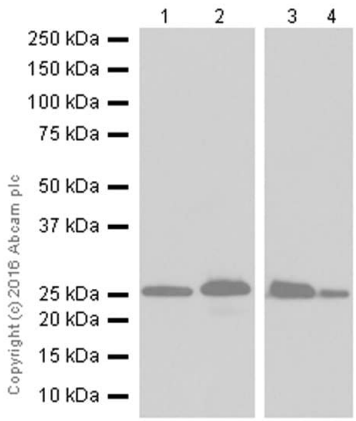
**Lane 1 :** Wild-type HAP1 whole cell lysate
**Lane 2 :** BCL2 knockout HAP1 whole cell lysate
**Lane 3 :** HeLa whole cell lysate
**Lane 4 :** THP-1 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 26 kDa
**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 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 ug/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 for 1 hour at room temperature before imaging.




Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

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

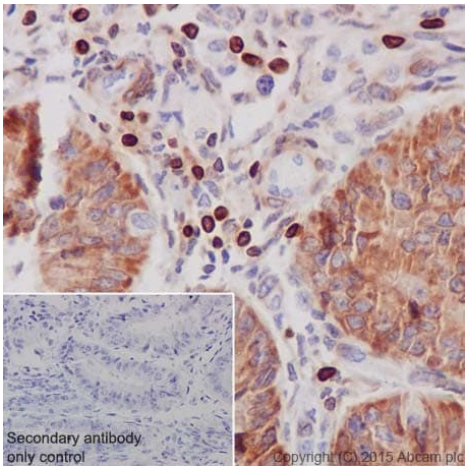
**Lane 1 :** NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate at 20 µg
**Lane 2 :** WEHI-3 (mouse leukemia cell line) whole cell lysate at 20 µg
**Lane 3 :** Mouse hippocampus at 10 µg
**Lane 4 :** Mouse heart at 10 µg

**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



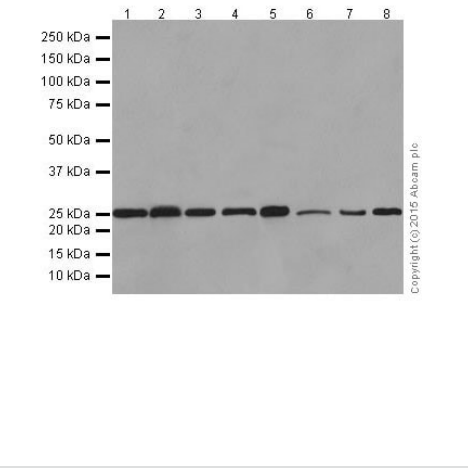

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [EPR17509] (ab182858)

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.




Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

**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.

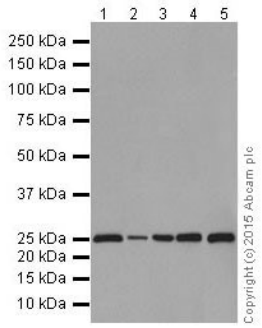
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

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.




Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

**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.

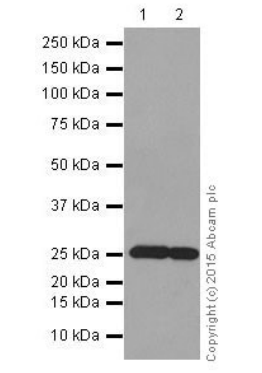
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

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.




Western blot - Anti-Bcl-2 antibody [EPR17509] (ab182858)

**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

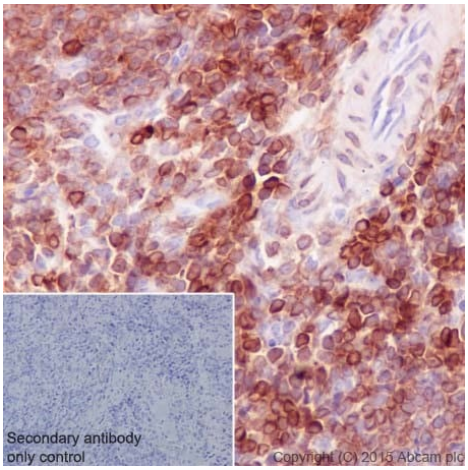
Developed using the ECL technique.

**Predicted band size:** 26 kDa

**Observed band size:** 26 kDa

**Exposure time:** 3 minutes

Blocking and diluting buffer was 5% NFDM /TBST.



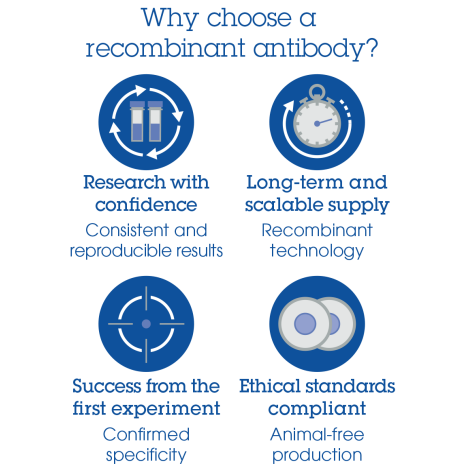

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [EPR17509] (ab182858)

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.




Anti-Bcl-2 antibody [EPR17509] (ab182858)



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