Product datasheet

**Anti-Bcl-2 antibody [E17] ab32124**

**Product name**
Anti-Bcl-2 antibody [E17]

**Description**
Rabbit monoclonal [E17] to Bcl-2

**Host species**
Rabbit

**Specificity**
Anti-Bcl-2 antibody [E17] (ab32124) recognises Bcl-2. It does not cross-react with other Bcl-2 family members.

**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 lymphoma 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**
Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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.

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.

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 x 10^-11 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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000. Detects a band of approximately 26 kDa (predicted molecular weight: 26 kDa). We recommend using ab182858 for murine samples.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>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.</td>
</tr>
<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/50.</td>
</tr>
</tbody>
</table>

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, 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**

**Images**

- **All lanes**: Anti-Bcl-2 antibody [E17] (ab32124) at 1/1000 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 - ab32124
observed at 26 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab32124 was shown to react with Bcl-2 in wild-type HeLa. Loss of signal was observed when knockout cell lysate ab263752 was used. Wild-type and Bcl-2 knockout samples were subjected to SDS-PAGE. ab32124 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 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.

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 10000 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) 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.

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.
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.

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 (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) 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

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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)

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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.

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

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.

Equilibrium disassociation constant (K_D)

Click here to learn more about K_D
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.

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