abcam

Product datasheet

Anti-Bcl-2 antibody ab7973

Overview

Product name Anti-Bcl-2 antibody

Description Rabbit polyclonal to Bcl-2

Host species Rabbit

Specificity Reacts with Bcl-2

Tested applications Suitable for: IHC-P, IHC-Fr, WB

Species reactivity Reacts with: Mouse, Rat, Cow, Human

Predicted to work with: Dog, Chinese hamster

Immunogen Synthetic peptide corresponding to Human Bcl-2 aa 1-18 (N terminal). Synthetic peptide:

AGRTGYDNREIVMKYIHY

, corresponding to N terminal amino acids 1-18 of Human Bcl-2.

Sequence:

AGRTGYDNREIVMKYIHY

Database link: P10415

(Peptide available as ab7983)

Run BLAST with
Run BLAST with

Positive control WB: HeLa, HL-60 and Jurkat lysate IHC: mouse cerebral cortex, cerebellum, hippocampus

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer Preservative: 0.1% Sodium azide

Constituents: 0.2% Gelatin, 99.7% PBS

Purity IgG fraction

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab7973 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
IHC-P	★★★ ★ (2)	1/100. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol. PubMed: 20800980In successful cases, the following protocol was used. Before the beginning of the procedure, slides should be put in a 200 ml Coplin jar filled with 10 mM citrate buffer and heated in a commercial microwave oven operating at a frequency of 2.45 GHz and 600 W power setting. After two heating cycles of 5
IHC-Fr		1/100. We have data to suggest that this antibody works on paraffin sections, however, we would recommed to use frozen sections and cannot guarantee IHC-P.
WB	★★★★ ☆ (7)	1/100.

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 lg 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 factorstimulated 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 starvationinduced 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.

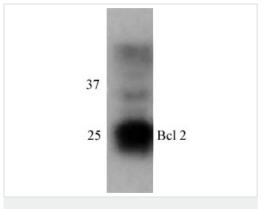
Monoubiquitinated by PARK2, leading to increase its stability. Ubiquitinated by SCF(FBXO10),

leading to its degradation by the proteasome.

Cellular localization

Mitochondrion outer membrane. Nucleus membrane. Endoplasmic reticulum membrane.

Images



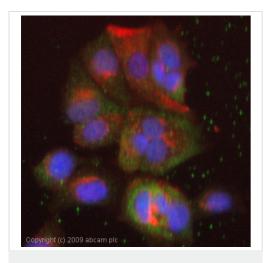
Western blot - Anti-Bcl-2 antibody (ab7973)

Anti-Bcl-2 antibody (ab7973) at 1/500 dilution + 25 ug HeLa whole cell lysate

Secondary

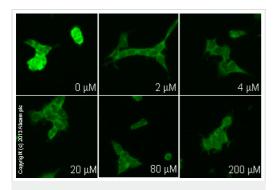
Goat anti rabbit HRP at 1/4000 dilution

This image is an edited version of an image submitted courtesy of an anonymous Abreview on 30 September 2005. We do not have any further information relating to this image.



Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 antibody (ab7973)

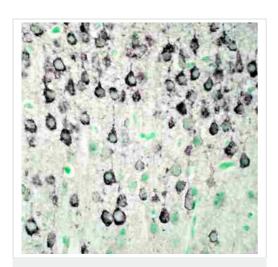
ICC/IF image of ab7973 stained MCF7 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab7973, 1 μ g/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit lgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 μ M.



Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 antibody (ab7973)

ab7973 staining Bcl-2 in HEK 293 cells treated with withaferin A from Withania somnifera (ab120644), by ICC/IF. Decrease in Bcl-2 expression with increased concentration of withaferin A from Withania somnifera, as described in literature.

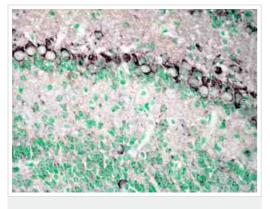
The cells were incubated at 37° C for 3h in media containing different concentrations of ab120644 (withaferin A from Withania somnifera) in DMSO, fixed with 100% methanol for 5 minutes at - 20° C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab7973 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A goat anti-rabbit DyLight 488 polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Bcl-2 antibody (ab7973)

Prof. Adalberto Merighi, Veterinary School, Univ. of Torino, Italy

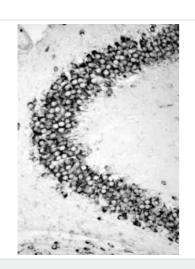
Fixation: 4% paraformaldehyde, paraffin embedding. Before the beginning of the procedure, slides are put in a 200 ml Coplin jar filled with 10 mM citrate buffer and heated in a commercial microwave oven operating at a frequency of 2.45 GHz and 600 W power setting. After two heating cycles of 5 minutes each, slides are allowed to cool at room temperature and thoroughly washed in phosphate buffered saline (PBS) pH 7.4 0.1 M. ABC peroxidase + Nickel intensification. Conterstaining Methyl green



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BcI-2 antibody (ab7973)

Prof. Adalberto Merighi, Veterinary School, Univ. of Torino, Italy

Sample treated using same protocol described in the above image.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Bcl-2 antibody (ab7973)

Prof. Adalberto Merighi, Veterinary School, Univ. of Torino, Italy

Sample treated using same protocol described in the above image, except with no counterstaining.

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