abcam

Product datasheet

Anti-Bcl-2 antibody [100/D5] ab692

KO VALIDATED

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Overview

Product name	Anti-Bcl-2 antibody [100/D5]
Description	Mouse monoclonal [100/D5] to Bcl-2
Host species	Mouse
Tested applications	**Suitable for:** WB, Flow Cyt, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Cow, Dog, Chinese hamster **Does not react with:** Rat
Immunogen	Synthetic peptide corresponding to Bcl-2 aa 41-54. Sequence: GAAPAPGIFSSQPG-Cys Database link: **P10415** Run BLAST with Run BLAST with
Positive control	IHC: Human tonsil ICC/IF: Human neuroblastoma (SK-N-SH cells) WB: HAP1 cells and HeLa cells Flow Cyt: Jurkat cells
General notes	This product was changed from ascites to tissue culture supernatant on 8th March 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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.

Storage buffer	pH: 7.30 Preservative: 0.09% Sodium azide Constituents: PBS, Tissue culture supernatant, 1% BSA Proprietary preservative that is not sodium azide or thimerosal, protein carrier.
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	100/D5
Myeloma	P3-NS1/1-Ag4-1
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab692 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

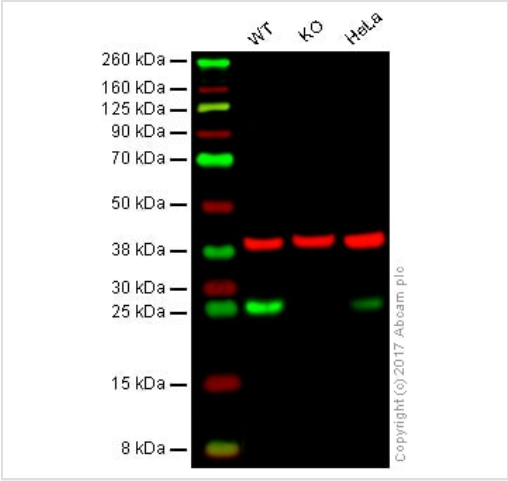
Application	Abreviews	Notes
WB	★★★★☆ **(7)**	Use at an assay dependent concentration. Predicted molecular weight: 26 kDa.
Flow Cyt		1/10. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration.
ICC/IF		Use a concentration of 5 µg/ml.

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, 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), leading to its degradation by the proteasome.
Cellular localization	Mitochondrion outer membrane. Nucleus membrane. Endoplasmic reticulum membrane.

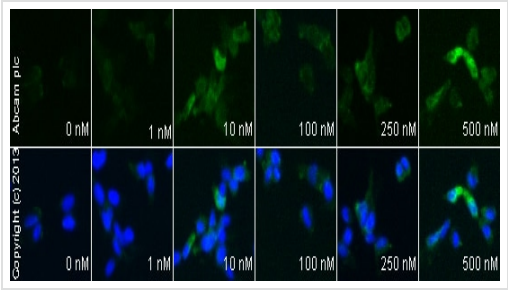
Images



Western blot - Anti-Bcl-2 antibody [100/D5] (ab692)

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)
Lanes 1 - 3: Merged signal (red and green). Green - ab692 observed at 26 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

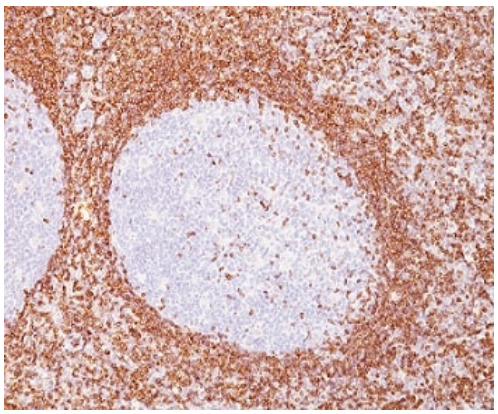
ab692 was shown to specifically react with BCL2 when BCL2 knockout samples were used. Wild-type and BCL2 knockout samples were subjected to SDS-PAGE. Ab692 and **ab181602** (Rabbit anti GAPDH loading control) were incubated overnight at 4°C at 500 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Bcl-2 antibody [100/D5] (ab692)

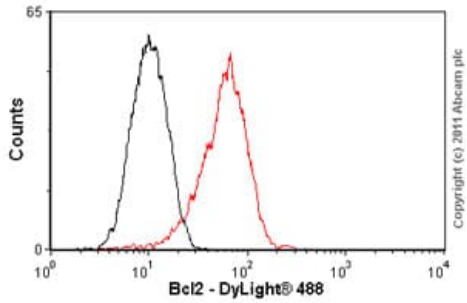
ab692 staining Bcl-2 in SK-N-SH cells treated with (R)-(-)-Deprenyl hydrochloride (Selegiline hydrochloride) (**ab120604**), by ICC/IF. Increase of Bcl-2 expression correlates with increased concentration of (R)-(-)-Deprenyl hydrochloride (Selegiline hydrochloride), as described in literature.
The cells were incubated at 37°C for 3h in media containing different concentrations of **ab120604** ((R)-(-)-Deprenyl hydrochloride (Selegiline hydrochloride)) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature 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 ab692 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A **anti-mouse DyLight 488** polyclonal antibody (**ab96879**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcl-2 antibody [100/D5] (ab692)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Bcl-2 with ab692 at 1/100 dilution. Samples were incubated with primary antibody for 30-45 minutes at RT.



Flow Cytometry - Anti-Bcl-2 antibody [100/D5] (ab692)

Overlay histogram showing Jurkat cells stained with ab692 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab692, 1/10 dilution) for 30 min at 22°C. The secondary antibody used was a goat **anti-mouse DyLight® 488** (IgG; H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

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