Anti-Bcl-2 Antibody [SZ10-03]

ET1603-11

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 26 kDa
Clone number:	SZ10-03

Description: Apoptosis is defined as a set of cascades which, when initiated, programs the cell to undergo lethal changes such as membrane blebbing, mitochondrial break down and DNA fragmentation. Bcl-2 is one among many key regulators of apoptosis, which are essential for proper development, tissue homeostasis, and protection against foreign pathogens. Human Bcl-2 is an anti-apoptotic, membrane-associated oncoprotein that can promote cell survival through protein-protein interactions with other Bcl-2 related family members, such as the death suppressors Bcl-xl, Mcl-1, Bcl-w, and A1 or the death agonists Bax, Bak, Bik, Bad, and Bid. The anti-apoptotic function of Bcl-2 can also be regulated through proteolytic processing and phospho-rylation. Bcl-2 may promote cell survival by interfering with the activation of the cytochrome c/Apaf-1 pathway through stabilization of the mitochondrial membrane. Mutations in the Bcl-2 gene can contribute to cancers where normal physiological cell death mechanisms are compromised by deregulation of the anti-apoptotic influence of Bcl-2.

Immunogen: Synthetic peptide within human Bcl-2 aa 40-90.

Positive control: HeLa cell lysate, Jurkat cell lysate, MCF7 cell lysate, HL-60 cell lysate, THP-1 cell lysate, A549, MCF-7, SH-SY5Y, human lymph nodes tissue, human kidney tissue, Jurkat.

Subcellular location: Mitochondrion outer membrane, Nucleus membrane, Endoplasmic reticulum membrane, Cytoplasm.

Database links: SwissProt: P10415 Human

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:800
IHC-P	1:50-1:4,000
FC	1:50-1:100
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

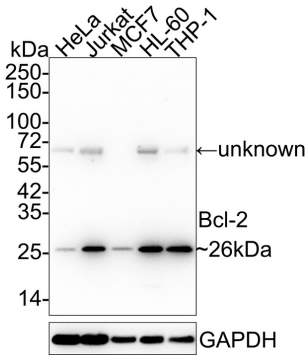

Fig1: Western blot analysis of Bcl-2 on different lysates with Rabbit anti-Bcl-2 antibody (ET1603-11) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: MCF7 cell lysate (20 µg/Lane)
Lane 4: HL-60 cell lysate (20 µg/Lane)
Lane 5: THP-1 cell lysate (20 µg/Lane)

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

Exposure time: 24 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1603-11) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

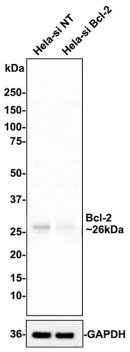

Fig2: Western blot analysis of Bcl-2 on different lysates with Rabbit anti-Bcl-2 antibody (ET1603-11) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si Bcl-2 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 31 seconds; ECL: merk

4-20% SDS-PAGE gel.

ET1603-11 was shown to specifically react with Bcl-2 in Hela-si NT cells. Weakened band was observed when Hela-si Bcl-2 sample was tested. Hela-si NT and Hela-si Bcl-2 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1603-11, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

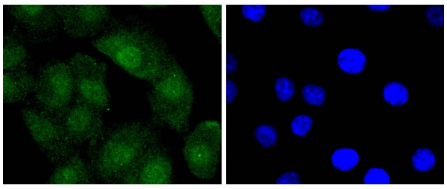

Fig3: ICC staining of Bcl-2 in A549 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-11, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

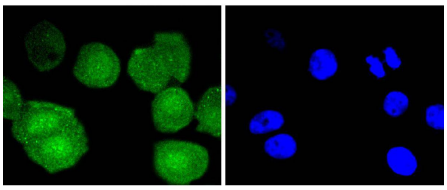

Fig4: ICC staining of Bcl-2 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-11, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

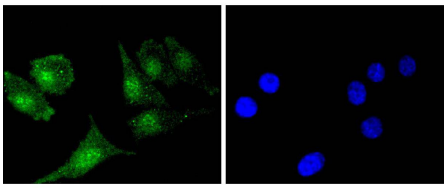

Fig5: ICC staining of Bcl-2 in SH-SY5Y cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-11, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

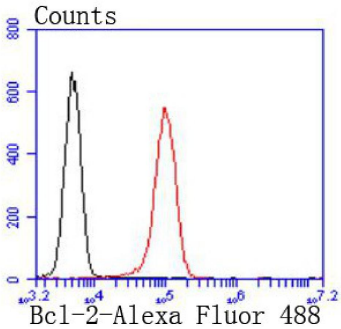

Fig6: Flow cytometric analysis of Bcl-2 was done on Jurkat cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1603-11, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

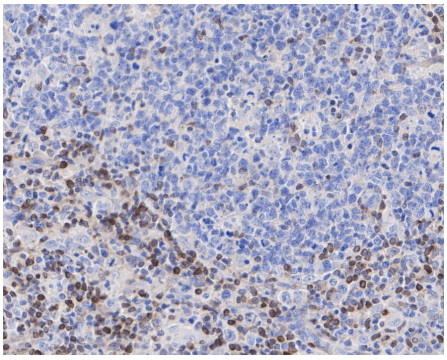

Fig7: Immunohistochemical analysis of paraffin-embedded human B-cell lymphoma tissue with Rabbit anti-Bcl-2 antibody (ET1603-11) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-11) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

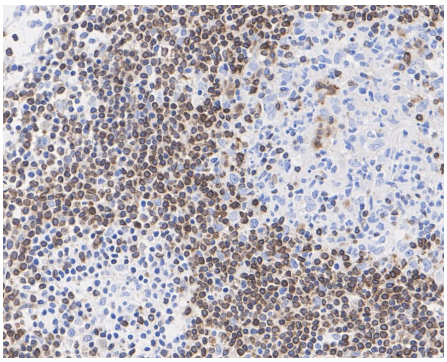

Fig8: Immunohistochemical analysis of paraffin-embedded human lymph node tissue with Rabbit anti-Bcl-2 antibody (ET1603-11) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-11) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Cao LH et al. Morphine, a potential antagonist of cisplatin cytotoxicity, inhibits cisplatin-induced apoptosis and suppression of tumor growth in nasopharyngeal carcinoma xenografts. Sci Rep 6:18706 (2016).
2. Chen B et al. Inhibition of miR-29c promotes proliferation, and inhibits apoptosis and differentiation in P19 embryonic carcinoma cells. Mol Med Rep 13:2527-35 (2016).