Anti-Bcl-2 Antibody [JF104-8]

ET1702-53

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 26 kDa
Clone number:	JF104-8

Description: 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: Recombinant protein within human Bcl-2 aa 1-230.

Positive control: HeLa cell lysate, Jurkat cell lysate, MCF-7 cell lysate, HL-60 cell lysate, THP-1 cell lysate, rat spleen tissue lysate, mouse spleen tissue lysate, Hela, A549, human tonsil tissue, human colon carcinoma tissue, mouse kidney tissue, human lung carcinoma tissue, Jurkat, human B-cell lymphoma tissue.

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

Database links: SwissProt: P10415 Human | P10417 Mouse | P49950 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:5,000
FC	1:500-1:1,000

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

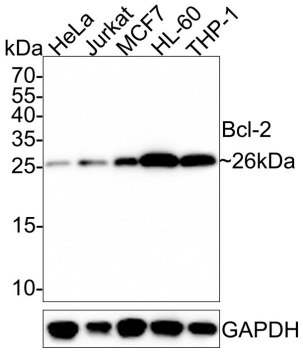

Fig1: Western blot analysis of Bcl-2 on different lysates with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: MCF7 cell lysate
Lane 4: HL-60 cell lysate
Lane 5: THP-1 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 1 minute;

15% 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 (ET1702-53) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

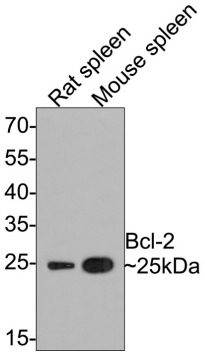

Fig2: Western blot analysis of Bcl-2 on different lysates with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/500 dilution.

Lane 1: Rat spleen tissue lysate
Lane 2: Mouse spleen tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 26 kDa
Observed band size: 25 kDa

Exposure time: 2 minutes;

12% 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 (ET1702-53) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

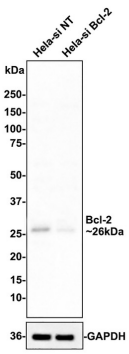

Fig3: Western blot analysis of Bcl-2 on different lysates with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/1,000 dilution.

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.

ET1702-53 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 (ET1702-53, 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.

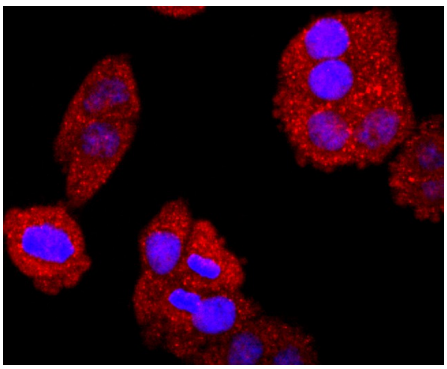

Fig4: ICC staining of Bcl-2 in Hela cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-53, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

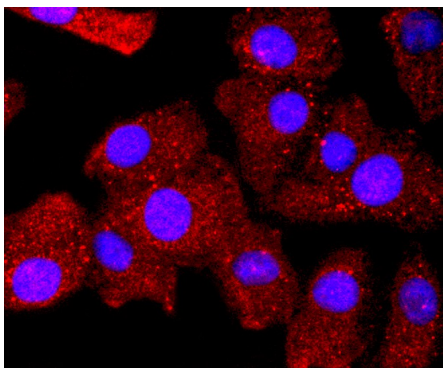

Fig5: ICC staining of Bcl-2 in A549 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-53, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

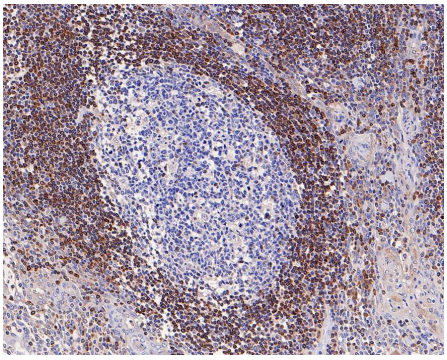

Fig6: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/5,000 dilution.

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 (ET1702-53) at 1/5,000 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.

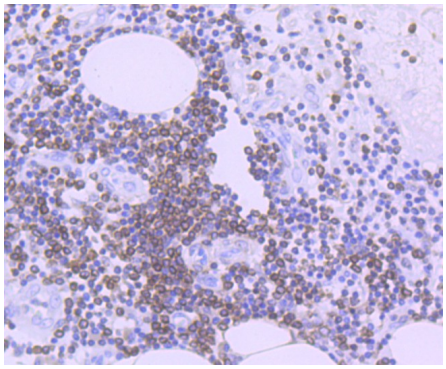

Fig7: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-Bcl-2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-53, 1/50) for 30 minutes 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.

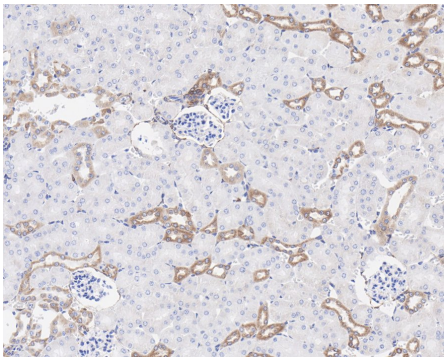

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/1,000 dilution.

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 (ET1702-53) 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.

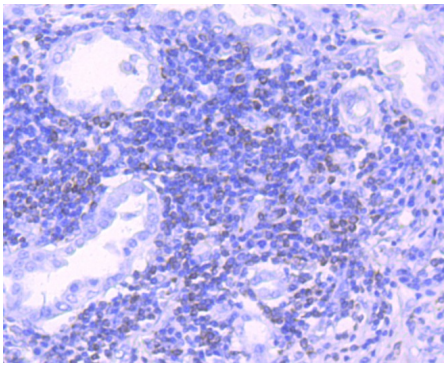

Fig9: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue using anti-Bcl-2 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-53, 1/50) for 30 minutes 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.

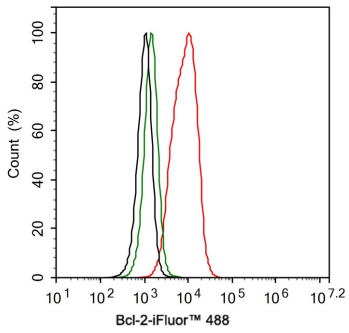

Fig10: Flow cytometric analysis of Jurkat cells labeling Bcl-2.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1702-53, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

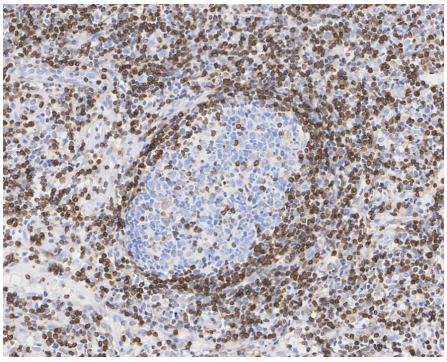

Fig11: Immunohistochemical analysis of paraffin-embedded human B-cell lymphoma tissue with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/5,000 dilution.

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 (ET1702-53) at 1/5,000 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.

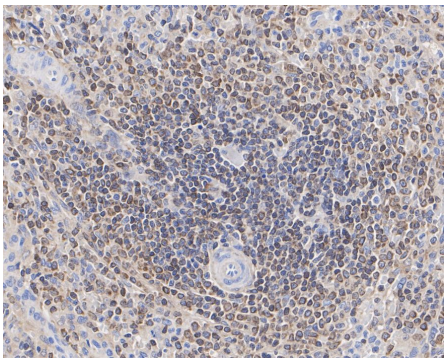

Fig12: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/1,000 dilution.

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 (ET1702-53) 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.

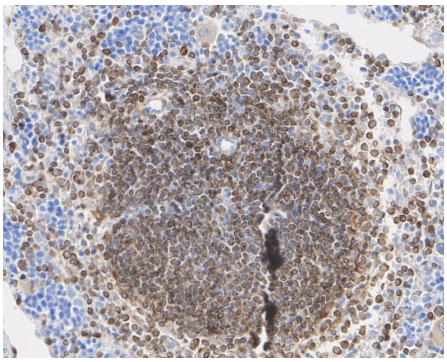

Fig13: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Bcl-2 antibody (ET1702-53) at 1/4,000 dilution.

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 (ET1702-53) at 1/4,000 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.

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).