Anti-Bcl-2 Antibody [PSH09-49]

HA723111

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 26 kDa
Clone number:	PSH09-49

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 1-233.

Positive control: HeLa cell lysate, 293T cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, C2C12 cell lysate, Mouse kidney tissue lysate, Rat spleen tissue lysate, Rat kidney tissue lysate, mouse spleen tissue, mouse kidney tissue, PC-12.

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	1:500
IHC-P	1:200
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

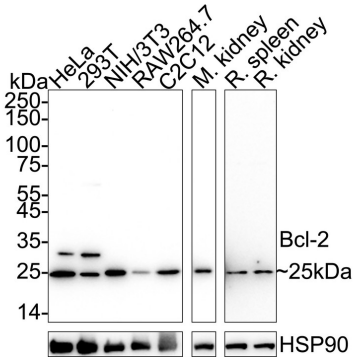

Fig1: Western blot analysis of Bcl-2 on different lysates with Rabbit anti-Bcl-2 antibody (HA723111) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: 293T cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: RAW264.7 cell lysate (20 µg/Lane)
Lane 5: C2C12 cell lysate (20 µg/Lane)
Lane 6: Mouse kidney tissue lysate (40 µg/Lane)
Lane 7: Rat spleen tissue lysate (40 µg/Lane)
Lane 8: Rat kidney tissue lysate (40 µg/Lane)

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

Exposure time: Lane 1-6: 20 seconds; Lane 7-8: 3 minutes; ECL: K1801;

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 (HA723111) at 1/2,000 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.

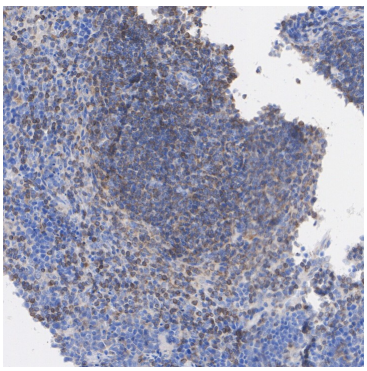

Fig2: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Bcl-2 antibody (HA723111) at 1/200 dilution.

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 (HA723111) at 1/200 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.

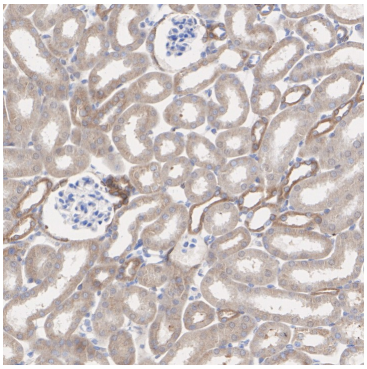

Fig3: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Bcl-2 antibody (HA723111) at 1/200 dilution.

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 (HA723111) at 1/200 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.

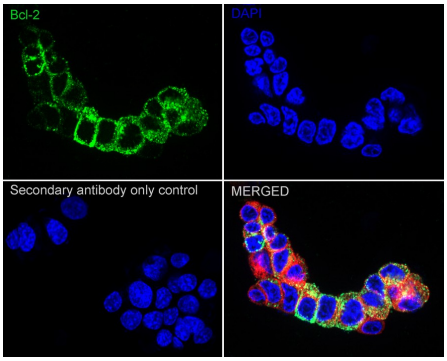

Fig4: Immunocytochemistry analysis of PC-12 cells labeling Bcl-2 with Rabbit anti-Bcl-2 antibody (HA723111) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Bcl-2 antibody (HA723111) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

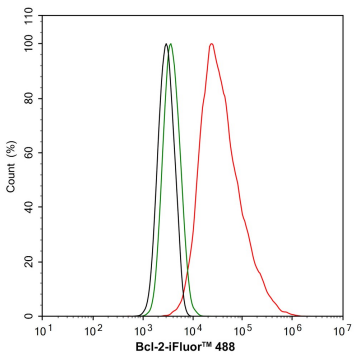

Fig5: Flow cytometric analysis of PC-12 cells labeling Bcl-2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723111, 1μg/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).

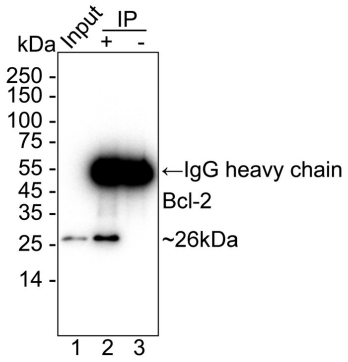

Fig6: Bcl-2 was immunoprecipitated from 0.2 mg NIH/3T3 cell lysate with HA723111 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723111 at 1/1,000 dilution. Mouse anti Rabbit IgG heavy chain (Fc) secondary antibody (M1003-7) at 1/10,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 cell lysate (input)
Lane 2: HA723111 IP in NIH/3T3 cell lysate
Lane 3: Rabbit IgG instead of HA723111 in NIH/3T3 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 14 seconds; ECL: K1801

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).