Anti-Bcl-XL Antibody [SZ3-03] - BSA and Azide free

HA750063

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 26 kDa
Clone number:	SZ3-03

Description:	It is a well-established concept in the field of apoptosis that relative amounts of pro- and anti-survival Bcl-2 family of proteins determine whether the cell will undergo cell death; if more Bcl-xL is present, then pores are non-permeable to pro-apoptotic molecules and the cell survives. However, if Bax and Bak become activated, and Bcl-xL is sequestered away by gatekeeper BH3-only factors (e.g. Bim) causing a pore to form, cytochrome c is released leading to initiation of caspase cascade and apoptotic events. While the exact signaling pathway of Bcl-xL is still not known, it is believed that Bcl-xL differs highly from Bcl-2 in their mechanism of inducing apoptosis. Bcl-xL is about ten times more functional than Bcl-2 when induced by the chemotherapy drug, Doxorubicin and can specifically bind to cytochrome C residues, preventing apoptosis. It can also prevent the formation of Apaf-1 and Caspase 9 complex by acting directly upon Apaf-1 rather than Caspase 9, as shown in nematode homologs.
Immunogen:	Synthetic peptide within Human Bcl-XL aa 37-86 / 233.
Positive control:	K-562 cell lysate, Jurkat cell lysate, B16-F1 cell lysate, RAW264.7 cell lysate, C6 cell lysate, Rat brain tissue lysate, K-562, human kidney tissue, mouse kidney tissue, rat kidney tissue, human colon carcinoma tissue, human breast carcinoma tissue.
Subcellular location:	Mitochondrion inner membrane, Mitochondrion outer membrane, Mitochondrion matrix, Cytoplasmic vesicle, Cytoplasm, Nucleus membrane.
Database links:	SwissProt: Q07817 Human | Q64373 Mouse | P53563 Rat
Recommended Dilutions:	
WB	1:1,000-1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000
FC	1:1,000
IP	Use at an assay dependent concentration.
Storage Buffer:	1*PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

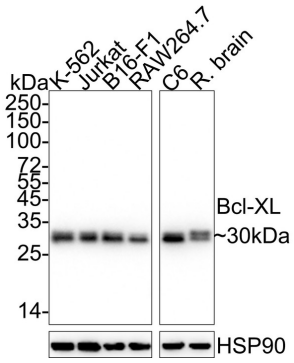

Fig1: Western blot analysis of Bcl-XL on different lysates with Rabbit anti-Bcl-XL antibody (HA750063) at 1/1,000 dilution.

Lane 1: K-562 cell lysate (10 µg/Lane)
Lane 2: Jurkat cell lysate (10 µg/Lane)
Lane 3: B16-F1 cell lysate (10 µg/Lane)
Lane 4: RAW264.7 cell lysate (10 µg/Lane)
Lane 5: C6 cell lysate (10 µg/Lane)
Lane 6: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 26 kDa
Observed band size: 30 kDa

Exposure time: 20 seconds; 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 (HA750063) at 1/1,000 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.

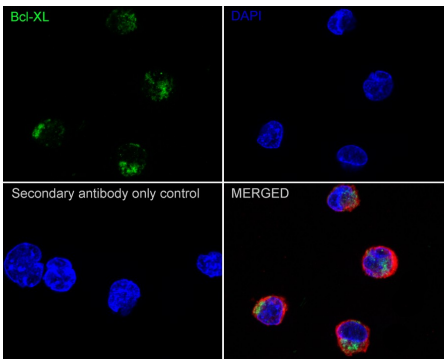

Fig2: Immunocytochemistry analysis of K-562 cells labeling Bcl-XL with Rabbit anti-Bcl-XL antibody (HA750063) at 1/100 dilution.

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-XL antibody (HA750063) at 1/100 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.

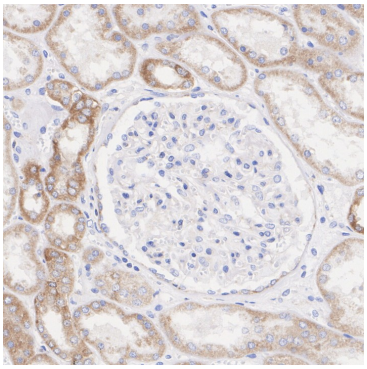

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Bcl-XL antibody (HA750063) at 1/1,000 dilution.

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 (HA750063) 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.

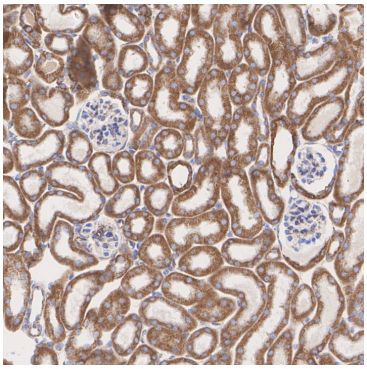

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Bcl-XL antibody (HA750063) at 1/1,000 dilution.

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 (HA750063) 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.

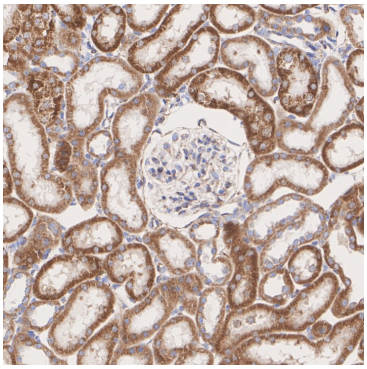

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Bcl-XL antibody (HA750063) at 1/1,000 dilution.

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 (HA750063) 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.

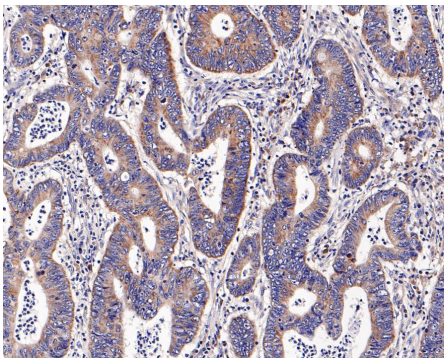

Fig6: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Bcl-XL antibody (HA750063) at 1/1,000 dilution.

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 (HA750063) 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.

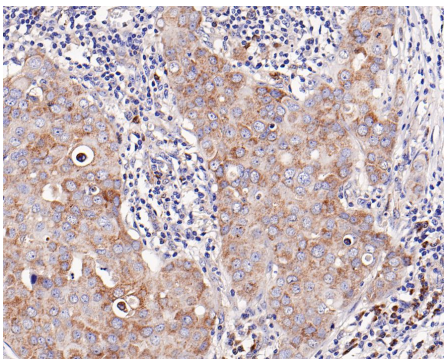

Fig7: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Bcl-XL antibody (HA750063) at 1/200 dilution.

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 (HA750063) 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.

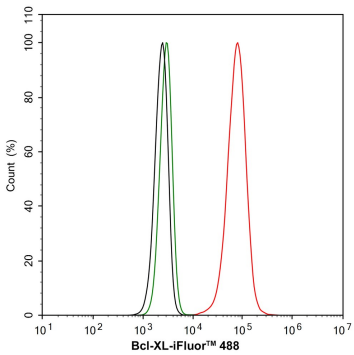

Fig8: Flow cytometric analysis of K-562 cells labeling Bcl-XL.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750063, 1/1,000) (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).

Background References

1. Cheng H Inhibiting CD146 by its Monoclonal Antibody AA98 Improves Radiosensitivity of Cervical Cancer Cells. Med Sci Monit 22:3328-33 (2016).
2. Wu J et al. Silencing of Kv1.5 Gene Inhibits Proliferation and Induces Apoptosis of Osteosarcoma Cells. Int J Mol Sci 16:26914-26 (2015).