

Anti-Bcl-2 Antibody [9F3-R]

HA601256



Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 26 kDa
Clone number:	9F3-R

Description: 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.

Immunogen: Synthetic peptide within human BCL2 aa 30-80.

Positive control: THP-1 cell lysate, HL-60 cell lysate, human tonsil tissue, human b-cell lymphoma tissue, THP-1.

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

Database links: SwissProt: P10415 Human

Recommended Dilutions:

WB	1:1,000
IHC-P	1:500
FC	1:1,000

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

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders: 0086-571-88062880

Technical: 0086-571-89986345

Service mail: support@huabio.cn

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Applications: WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of Bcl-2 on different lysates with Mouse anti-Bcl-2 antibody (HA601256) at 1/1,000 dilution.

Lane 1: THP-1 cell lysate

Lane 2: HL-60 cell lysate

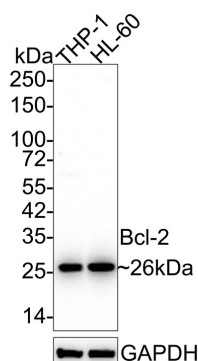
Lysates/proteins at 20 µg/Lane.

Predicted band size: 26 kDa

Observed band size: 26 kDa

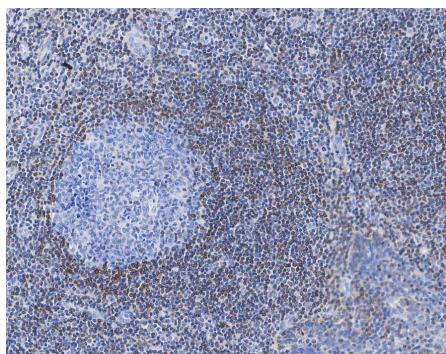
Exposure time: 1 minute 40 seconds;

4-20% SDS-PAGE gel.



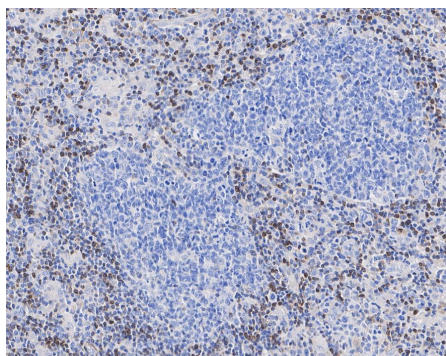
Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601256) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Mouse anti-Bcl-2 antibody (HA601256) at 1/500 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 ddH₂O and PBS, and then probed with the primary antibody (HA601256) at 1/500 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 human b-cell lymphoma tissue with Mouse anti-Bcl-2 antibody (HA601256) at 1/500 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 ddH₂O and PBS, and then probed with the primary antibody (HA601256) at 1/500 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.

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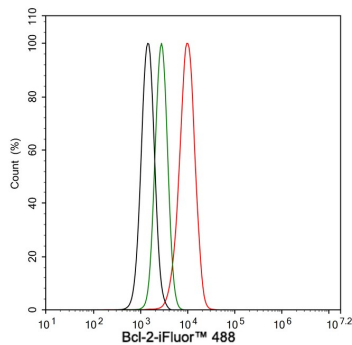


Fig4: Flow cytometric analysis of THP-1 cells labeling Bcl-2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA601256, 1 μ g/mL) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Background References

1. Yin X-M. et. al. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature* 369:321-323 (1994).
2. Naumovski L. et. al. The p53-binding protein 53BP2 also interacts with Bcl2 and impedes cell cycle progression at G2/M. *Mol Cell Biol* 16:3884-3892 (1996).

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