

Affinity-Purified Goat Anti-Bcl-2

ORDERING INFORMATION

Catalog Number: AF810

Lot Number: BJW02

Size: 100 µg

Storage: -20° C

Specificity: human Bcl-2, mouse Bcl-2

Immunogen: aa 1 - 208 of Bcl-2

Host: Bcl-2 specific goat IgG

Applications: Western blot
Immunoprecipitation
Immunohistochemistry

Preparation

Goats were immunized with recombinant mouse Bcl-2, aa 1 - 208, which lacks the C-terminal mitochondrial targeting sequence. Immunogen was tagged with 6X-histidines at the C-terminal. Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute the antibody in 100 µL of PBS containing 0.02% NaN₃.

Storage

Avoid repeated freezing and thawing by aliquoting smaller portions of the reconstituted antibody into Eppendorf tubes and storing at -20° C.

Specificity

The antibody is known to react with human Bcl-2 and mouse Bcl-2.

Western blot

An antibody concentration of 1.0 µg/mL is recommended.

Immunoprecipitation

3 µg of antibody per immunoprecipitation of Bcl-2 from 3 x 10⁶ MNFS60 cells is recommended.

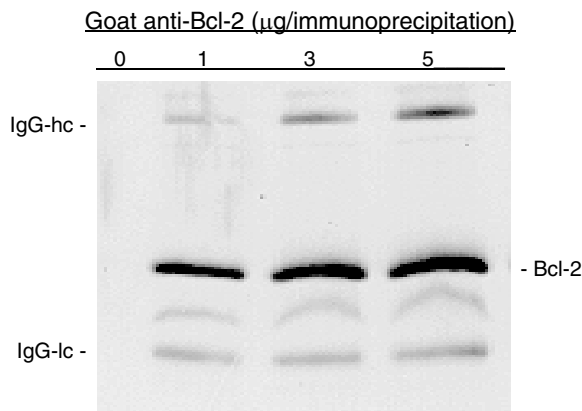
Immunohistochemistry

This antibody will detect Bcl-2 in mouse immune system cells. The working dilution is 15 µg/mL. Detection may be done using fluorescence immunocytochemistry.

Optimal dilutions should be determined by each laboratory for each application.

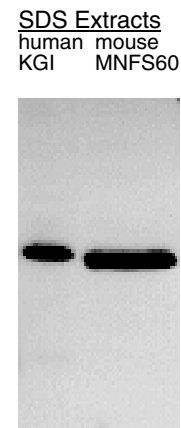
For immunohistochemistry images, refer to our website at http://www.rndsystems.com/asp/c_immunohistochemistry_add.asp

A. Bcl-2 was immunoprecipitated from 3 x 10⁶ mouse MNFS60 cells, using 0, 1, 3 and 5 µg/mL of goat anti-Bcl-2. One fourth of the immunoprecipitate was loaded on the gel.



Film was exposed for 2 seconds.

B. SDS extracts from 3 x 10⁶ human KGI and 1 x 10⁶ mouse MNFS60 cells were also electrophoresed on the gel. After transfer to membranes, the samples were blotted with anti-Bcl-2. Methods are described in the attached protocol.



Film was exposed for 15 seconds.

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1-800-343-7475

Protocols for Immunoprecipitation and Immunoblotting with Goat anti-Bcl-2

Western blotting

<u>Blotting buffer</u>	<u>Blocking solution</u>	<u>Antibody solution</u>
25 mM Tris, pH 7.5	5% nonfat dry milk in blotting buffer	2% nonfat dry milk in blotting buffer
0.15 M NaCl	pH 7.5	pH 7.5
0.05% Tween 20		

1. Transfer the electrophoresed proteins to Immobilon filters (Millipore) and incubate the membrane for 1 hour at room temperature in blocking solution.
2. Incubate the membrane for 1 hour in antibody buffer containing 1.0 µg/mL Goat anti-Bcl-2.
3. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
4. Incubate the membrane for 1 hour in antibody buffer containing a 1:2,000 dilution of HRP-conjugated Rabbit anti-goat IgG (Zymed).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detection was with ECL Reagent (Amersham).

Cell lysates for Western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Immunoprecipitation

<u>Wash buffer</u>	<u>Cell lysis buffer</u>
20 mM Tris, pH 7.5	wash buffer containing:
0.15 M NaCl	0.25 mM phenylmethylsulfonyl fluoride
2 mM EDTA	1 µg/mL aprotinin
1% Triton X-100	1 µg/mL leupeptin
0.02% NaN_3	1 µg/mL chymostatin
10 mM NaF	
1 mM sodium ortho-vanadate	

Cell lysis for immunoprecipitation: Cells are rinsed three times with cold phosphate buffered saline and cell protein is extracted by solubilization at 1×10^6 - 1×10^7 cells per mL of cold cell lysis buffer. The extraction mixtures are rocked at 4° C for 30 - 60 minutes. Lysates are then centrifuged at 3,000 x g for 5 minutes to remove insoluble material. One mL of cell lysate is precleared by incubation for 5 minutes with 10 µL of a 20% suspension of Protein G (Sigma). Protein G is pelleted by centrifugation at 12,000 x g for 0.5 minutes in an Eppendorf centrifuge, the supernatant is transferred to a new tube, and the preclearing is repeated one or more times. Protein G had been washed three times with wash buffer before being added to the extracts.

Immunoprecipitation: Goat anti-Bcl-2 is added to the 1 mL extract and the mixture is rocked for 1 hour at 4° C. Protein G (Sigma, 50 µL of a 20% suspension) is then added and the mixture is rocked in the cold for an additional 0.5 hours. The Protein G-absorbed complexes are centrifuged for 0.5 minutes in an Eppendorf centrifuge, resuspended in extraction buffer by trituration with a glass Pasteur pipet, and then repelleted. The complexes are washed a total of three times with extraction buffer, and then suspended in phosphate buffered saline and transferred to a new tube before the final centrifugation. The washed pellet is suspended in 25 - 50 µL of 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) by vortexing and then incubated for 3 minutes in a boiling water bath. Protein G is pelleted and the supernatant is loaded on a polyacrylamide gel.