

Affinity-Purified Rabbit Anti-human/mouse Bax

ORDERING INFORMATION

Catalog Number: AF820

Lot Number: BOZ05

Size: 30 µg

Storage: -20° C

Specificity: human Bax, mouse Bax

Immunogen: aa 12 - 33 of human Bax

Host: Bax specific rabbit IgG

Applications: Western blot
Immunohistochemistry

Preparation

Rabbits were immunized with the KLH coupled synthetic peptide, GPTSSEQIMKTGALLLQGFIQDC (corresponding to amino acids 12 - 33 of human Bax). Cysteine was added to the carboxyl-terminal for coupling to KLH and for coupling to an affinity matrix. Polyclonal antibody was affinity-purified on a column derivatized with the peptide. The sequence used for immunization differs significantly from the sequences of the corresponding region of other members of the Bcl-2 family.

Formulation

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

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 in a manual defrost freezer.

Specificity

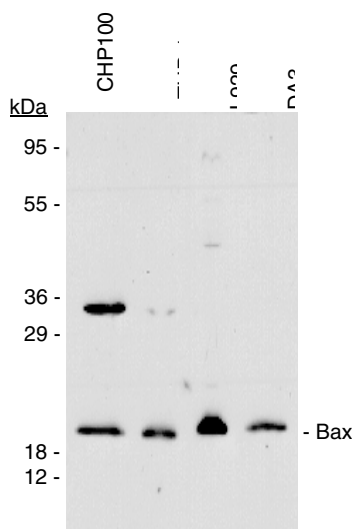
The antibody is known to react with human and mouse Bax.

Western blot

An antibody concentration of 0.3 µg/mL is recommended.

Immunohistochemistry

The antibody will detect Bax in paraffin-embedded human tissue sections. The working dilution for 5 - 15 µm thick sections is 15 µg/mL. For chromogenic detection of labeling it is recommended to use R&D Systems Cell and Tissue Staining Kits (CTS Series).



Film was exposed for
15 seconds.

Immunoblots of SDS-extracts from 2×10^5 human (CHP100 and THP-1) and mouse (L929 and DA3) cells. Extracts were blotted with 0.3 µg/mL anti-Bax. Incubation with anti-Bax was overnight at 4° C and detection was by the ECL procedure (Amersham). The identity of the 35 kDa band detected in human cell extracts is unknown.

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R&D Systems, Inc.
1-800-343-7475

Protocols for Immunoblotting

Western blotting

Blotting buffer

25 mM Tris, pH 7.5
0.15 M NaCl
0.05% Tween[®] 20

Blocking solution

5% nonfat dry milk in blotting buffer
pH to 7.5

Antibody solution

2% nonfat dry milk in blotting buffer
pH to 7.5

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 overnight at 4° C in antibody buffer containing 1.0 µg/mL rabbit anti-human/mouse Bax.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer.
4. Incubate the membrane for 1 hour at room temperature in antibody buffer containing a 1:2,000 dilution of HRP-conjugated Protein A (Amersham).
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.

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