Protein G-purified Anti-human Bax

ORDERING INFORMATION

Catalog Number: AB820

Lot Number: CCJ0109021

Size: 400 µg

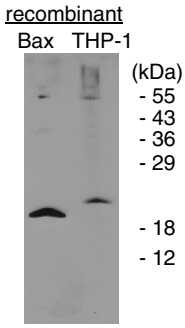
Storage: -20° C

Specificity: human Bax

Immunogen: human Bax

Ig Type: Total goat IgG

Applications: Western blot
Immunohistochemistry



Immunoblots of 1 ng of recombinant Bax that was used as an immunogen and of SDS-extracts from 2 x 10^5 human THP-1 cells. Cells were electrophoresed on 15% gels and immunoblotting was with 4.0 µg/mL Protein G-purified anti-Bax. Incubation with anti-Bax was overnight at 4° C and detection was by the ECL procedure (Amersham). An exposure of one minute is shown.

Preparation
Goats were immunized with the purified, recombinant human Bax that was missing the carboxyl terminal mitochondrial targeting sequence. Polyclonal antibody was purified on a column derivatized with Protein G.

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% NaN_3.

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 detects human Bax. The antibody does not detect mouse Bax.

Applications
Western blot - An antibody concentration of 4.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting buffer	Blocking solution	Antibody solution
25 mM Tris, pH 7.5	5% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in blotting buffer	in blotting buffer
0.05% Tween® 20	pH to 7.5	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 solution containing 4.0 µg/mL goat anti-Bax.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane for 1 hour at room temperature in antibody solution 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 x 10^6 - 1 x 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.

Immunohistochemistry - This antibody will detect Bax in cells and tissues. The working dilution is 15 µg/mL. For chromogenic detection of labeling, use R&D Systems Cell and Tissue Staining Kits (CTS Series).

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

R&D Systems, Inc.
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