

Anti-Bax, N-terminal
(rabbit polyclonal IgG)
Catalog # 06-499
Lot # 17692

Immunogen: Synthetic peptide [MDGSGEQPRGGGPTSSEQIMK-C] corresponding to amino acid residues 1-21 of human Bax with a cysteine residue added on the C-terminus for conjugation to KLH.

Specificity: Specific for Bax α p20 and β p23.

Species Cross-reactivity: Human and mouse.

Formulation: 200ng protein G purified rabbit IgG in 200 μ l of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl with 0.05% sodium azide. Frozen solution.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

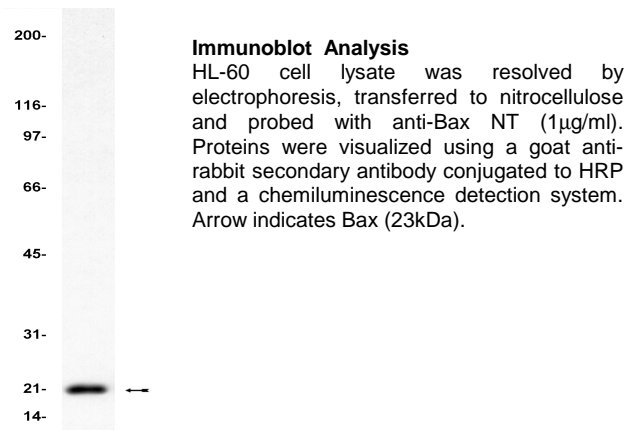
Quality Control Testing

Western Immunoblot Analysis: 0.5-2 μ g/ml of this lot detected Bax (~23kDa) in 20 μ g of cell lysates from human HL-60 cells.

Immunoprecipitation: 4 μ g of this lot immunoprecipitated Bax from 500 μ g of a mouse ABE 8 1/2 and human HL-60 lysates.

Additional Research Applications

Immunocytochemistry: 10 μ g/ml of previous lots detected Bax in mouse ABE 8 1/2 cells fixed in 1% paraformaldehyde, followed by permeabilization with 100% methanol for 60 seconds.



Background: The relative expression of members of the *bcl-2/ced-9* gene family apparently regulates the cellular decision to execute apoptosis in response to specific signals or damage.¹ Studies indicate that elevated levels of the Bcl-2 protein prolong cell survival.¹ In contrast, elevated levels of a homologous protein, Bax, promote cell death. Evidence now indicates that Bcl-2 and Bax form homo- and heterodimers.² This observation suggests that the ratio of Bcl-2 to Bax may comprise a molecular rheostat for survival.² Interestingly, p53, which is required for apoptosis induced by ionizing radiation, transactivates reporter gene expression driven by the *bax* promoter, suggesting that *bax* may be a primary response gene of p53.³

References:

1. Reed, J.C., *J. Cell Biol.* **124**: 1-6, 1994
2. Oltvai, Z., *et al.*, *Cell* **74**: 609-619, 1993.
3. Miyashita, T. and J.C. Reed, *Cell* **80**: 293-299, 1995.

Application References:

1. Otter, I., *et al.*, *J. Biol. Chem.* **273**: 6110-6120, 1998.
2. Monney, L., *et al.*, *J. Biol. Chem.* **273**: 6121-6131, 1998.
3. Rosse, T., *et al.*, *Nature* **391**: 496-499, 1998.

Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/ml of a-Bax NT**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit IgG linked to horseradish peroxidase, 1:3000 dilution, was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Before beginning the immunoprecipitation, dilute the cell lysate to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **4µg of a-Bax, NT** to 500µg-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl of washed Protein G or A agarose bead slurry (50µl packed beads).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50µl 2X Laemmli sample buffer and boil for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant, or the agarose beads can then be frozen for later use and reboiled for 5 minutes prior to SDS-PAGE.