

**Anti-Bax, N-terminal**  
(rabbit polyclonal IgG)  
Catalog # 06-499  
Lot # 18416

**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  $\alpha$  p20 and  $\beta$  p23.

**Species Cross-reactivity:** Human and mouse.

**Formulation:** **200ng** protein G purified rabbit IgG in **200 $\mu$ l** of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide containing 30% glycerol. 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**

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**Quality Control Testing**

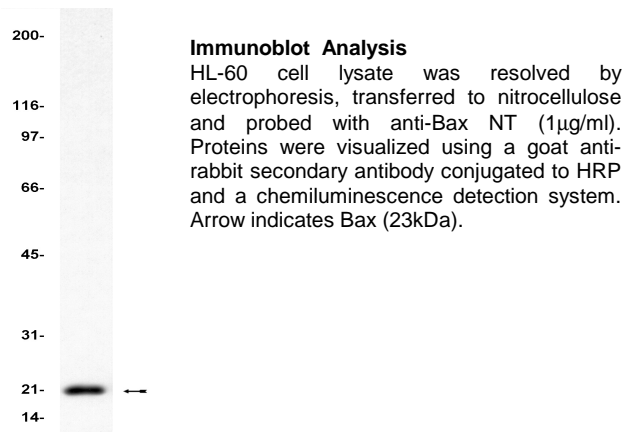
Western Immunoblot Analysis: 1-2 $\mu$ g/ml of this lot detected Bax (~23kDa) in 20 $\mu$ g of cell lysates from human HL-60 cells.

Immunoprecipitation: 4 $\mu$ g of this lot immunoprecipitated Bax from human HL-60 lysate; a previous lot immunoprecipitated Bax from 500 $\mu$ g of mouse ABE 8 1/2.

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**Additional Research Applications**

Immunocytochemistry: 10 $\mu$ 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.



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**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.<sup>1</sup> Studies indicate that elevated levels of the Bcl-2 protein prolong cell survival.<sup>1</sup> In contrast, elevated levels of a homologous protein, Bax, promote cell death. Evidence now indicates that Bcl-2 and Bax form homo- and heterodimers.<sup>2</sup> This observation suggests that the ratio of Bcl-2 to Bax may comprise a molecular rheostat for survival.<sup>2</sup> 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.<sup>3</sup>

**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 Na<sub>3</sub>VO<sub>4</sub>; 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 **1-2ng/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 **4ng 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 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.