



cell signaling solutions

## Certificate of Analysis

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**Anti-Bax, NT**  
(rabbit polyclonal IgG)  
Catalog # 06-499  
Lot # 24156

**Immunogen:** Synthetic peptide, [MDGSGEQP-RGGGPTSSEQIMK-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:** 200 $\mu$ g protein A purified rabbit IgG in 230 $\mu$ l of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the original vial prior to removing the cap.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing

**Immunoblot Analysis:** 0.5-2 $\mu$ g/ml of this lot detected Bax 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.

### 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.



#### Immunoblot Analysis

Representative blot from a previous lot. HL-60 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Bax, NT (1 $\mu$ g/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Bax (~23kDa).

### References:

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

### Application References:

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

### 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; 1 $\mu$ g/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 (Catalog # 20-200), PBS-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 $\mu$ g/ml of anti-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** HRP conjugated, Catalog # 12-348, 1:5000 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 1 $\mu$ g/ $\mu$ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4 $\mu$ g of anti-Bax, NT** to 500 $\mu$ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu$ l (50 $\mu$ l packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125).
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 60 $\mu$ l 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 be frozen for later use and reboiled for 5 minutes prior to SDS-PAGE.