

Certificate of Analysis

Anti-Bax, NT
(rabbit polyclonal IgG)
Catalog # 06-499
Lot # 31947

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 α p20 and β p23.

Species Cross-reactivity: Human and mouse.

Formulation: 200 μ g protein A purified rabbit IgG in 200 μ l of 70% storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide) and 30% glycerol.

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

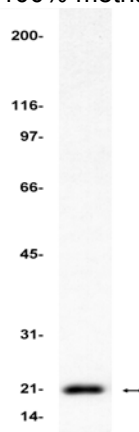
Quality Control Testing

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

Immunoprecipitation: 4 μ g of previous lots immunoprecipitated Bax from human HL-60 and mouse ABE 8 1/2 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.³³



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 μ g/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Bax (~23kDa).

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.
4. Guillermet, J., *et al.*, *Proc. Natl. Acad. Sci. USA* **100**: 155-160, 2003.
5. Lasham, A., *et al.*, *J. Biol. Chem.* **278**: 35516-35523, 2003.

General References:

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

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 μ g/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 (Catalog # 20-200), (PBS-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ 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 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4 μ g of anti-Bax, NT** to 500 μ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ 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 μ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.