upstate biotechnology

Certificate of Analysis

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Anti-Human Bax, N-terminal

(rabbit polyclonal IgG)
Catalog # 06-499
Lot # 15670

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: Mouse.

Formulation: **200mg** protein G purified rabbit IgG in **200ml** of 0.1M Tris-glycine, pH 7.0, 0.15M NaCl with 0.05% sodium azide. Frozen solution.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing and Research Applications

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

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

Immunocytochemistry: 10µg/ml of this lot 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 Reed J.C., 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.

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_3VO_4; 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 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **2mg/ml of a-Human Bax, N-terminal**, 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 **4mg of ?-Human Bax, N-terminal** 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.

Immunocytochemistry

1. Plate approximately 200µl of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO_2 incubator.
2. Wash the cells three times for 5 minutes with PBS. Do not shake cells.
3. Add fix (ice-cold 1% paraformaldehyde) in PBS for 30 minutes at room temperature.
4. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
5. Permeabilize cells with ice-cold 100% methanol for 60 seconds.
6. Wash cells with PBS twice for 15 minutes. Do not shake.
7. Add 400µl of 8% albumin in PBS and incubate for 30 minutes at room temperature.
8. Wash the cells with PBS, for 15 minutes.
9. Incubate the cells with **10mg/ml a-Human Bax, N-terminal** in 1% albumin in PBS and incubate overnight at 4°C.
10. Wash the cells twice with PBS, for 5 minutes.
11. Incubate the cells with a **1:100 dilution of goat anti-rabbit IgG** fluorescein conjugated secondary antibody in PBS for 1 hour at room temperature.
12. Wash the cells three times with PBS, for 5 minutes.
13. Examine the cells under a fluorescent microscope.