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upstate®
cell signaling solutions
a Serologicals® Company

Certificate of Analysis

Anti-Bak, NT
(rabbit polyclonal IgG)
Catalog # 06-536
Lot # 31936

Immunogen: Synthetic peptide (SEEQVAQQDTE-EVFRSC) corresponding to amino acid residues 23-38 of human Bak with a cysteine residue added to the C-terminus for conjugation to KLH.

Specificity: Specific for Bak, Mr 30kDa.

Species Cross-Reactivity: Human and mouse.

Formulation: **200µg** of protein A purified rabbit IgG in **200µl** of storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide). Frozen liquid.

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.**

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

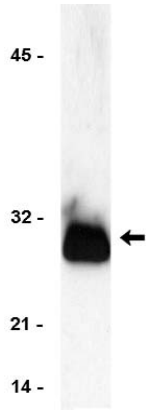
Immunoblot Analysis: 0.5-2µg/ml of this lot detected Bak in 20µg of human A431 cell lysate. A previous lot detected Bak in 20µg of human A431 and mouse 3T3 cell lysates.

Included Positive Antigen Control: Catalog # 12-301, non-stimulated human A431 cell lysate. **Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

Immunohistochemistry: 5µg/ml of a previous lot detected Bak in paraffin-embedded rat kidney tissue.

Additional Research Applications

Immunoprecipitation: 4µg of a previous lot immunoprecipitated Bak from 500µg mouse 3T3 cell lysate.



Immunoblot Analysis: Representative blot from a previous lot. Non-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Bak, NT (0.5µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Bak (~30kDa).

References:
1. Farrow, S.N., *et al.*, Nature **374**: 731-736, 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 EDTA; 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 (Catalog # 20-200), (PBS-MLK) for 60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2μg/ml of anti-Bak, NT**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C overnight or 3 hours at room temperature.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, 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 500μg-1mg of cell lysate to roughly 1μg/μl total cell protein in a microcentrifuge tube with PBS.
2. Add **4μg of anti-Bak, NT** to the cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100μl (50μl packed beads) of washed Protein A agarose bead slurry
5. (Catalog # 16-125).
6. Gently rock the reaction mixture at 4°C for 2 hours.
7. 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.
8. Resuspend the agarose beads in 60μl 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.