



cell signaling solutions

## Certificate of Analysis

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**Anti-Bak, NT**  
(rabbit polyclonal IgG)  
Catalog # 06-536  
Lot # 27749

**Immunogen:** Synthetic peptide (SEEQVAQQDTE-EVFRSC) corresponding to amino acid residues 23-37 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.

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

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

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

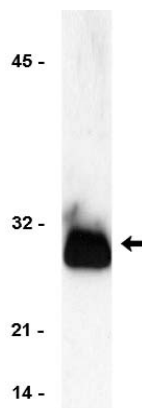
### Quality Control Testing

**Immunoblot Analysis:** 0.5-2µg/ml of this lot detected Bak in 20µg of human A431 cell lysate. 0.5-2µg/ml of 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 per 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 this antibody can detect Bak in paraffin-embedded rat kidney tissue.

**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; 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 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 $\mu$ g/ml of anti-Bak, NT**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C overnight.
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 500 $\mu$ g-1mg of 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-Bak, NT** to the 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 then be frozen for later use and reboiled for 5 minutes prior to SDS-PAGE.