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Certificate of Analysis

Anti-Bcl-3
(rabbit polyclonal IgG)
Catalog # 06-415
Lot # 21065

Immunogen: Fusion protein containing amino acid residues 290-421 of human Bcl-3.

Species Cross-Reactivity: Human.

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 product, centrifuge vial after thawing and prior to removing cap.

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

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

Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected Bcl-3 from 20µg of a Bcl-3 transfected Cos-1 cell lysate. This antibody does not effectively blot endogenous levels of Bcl-3 from RIPA cell lysates.

Immunoprecipitation: 4µg of this lot co-immunoprecipitated NF-κB, p52 subunit from a Jurkat RIPA cell lysate as detected using anti-NF-κB, p52 (Catalog # 05-361).

Additional Research Applications

Immunocytochemistry: This antibody has been reported to stain Bcl-3 in fixed cells.^{1,2}

Application References:

1. Bours, *et al.*, Cell **72**: 729-739, 1993.
2. Franzoso, *et al.*, EMBO J. **12**: 3893-3901, 1993.

Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a Bcl-3 transfected 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 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 (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/ml of anti-Bcl-3**, 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 IgG linked to horseradish peroxidase, 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 1µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **4µg of anti-Bcl-3** 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 A agarose bead slurry (50µl packed beads), 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 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.