

Anti-Human Bcl-3
(rabbit polyclonal IgG)

Catalog # 06-415

Lot # 13484

Background: This proto-oncogene is involved in 14; 19 transposition. It could be a transcriptional activating factor.

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

Physical Form: Frozen liquid.

Formulation: 200µg protein A purified IgG in 63µl 0.1M Tris-glycine, pH 7.4.

Species Cross Reactivity: Unknown.

Storage and Shelf Life: 2 years at -20°C.

6 months at 4°C.

Avoid repeated freezing and thawing.

Included Positive Antigen Control for Western Immunoblot Analysis: Catalog #12-303, jurkat cell lysate.
Use 20µg per lane.

References:

¹Bours, *et. al.*, Cell **72**: 729-739, (1993).

²Franzoso, *et. al.*, EMBO J. **12(10)**: 3893-3901, (1993).

FOR RESEARCH USE ONLY.
NOT FOR USE IN HUMANS.

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Quality Control Testing and Research Applications

Western Immunoblot Analysis: Used at 1-4 μ g/ml, this lot of antibody detected Bcl-3 protein and another protein (~120kDa) in human Jurkat cell lysates (Catalog #12-303).

Immunoprecipitation: Use 4 μ g to coimmunoprecipitate NF κ B with Bcl-3 from Jurkat cell lysates.

Immunocytochemistry: It has been reported elsewhere for use in immunocytochemistry staining on fixed cells^[1,2].

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 α -Human Bcl-3** 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 of washed Protein A agarose bead slurry (50 μ l 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 50 μ 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.

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; 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 (MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1-4 μ g/ml of α -Human 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, 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).