

**PN IM3526 Polyclonal Antibody Bad**

<b>Form</b>	Unconjugated	<b>Clone</b>	Polyclonal
<b>Quantity</b>	0.2 mg	<b>Isotype</b>	Not applicable
<b>Presentation</b>	Liquid 0.1 mL	<b>Species</b>	Rabbit

For Research Use Only. Not For Use In Diagnostic Procedures.

**SPECIFICITY**

The Bcl-2 related proteins can inhibit (Bcl-x<sub>L</sub> and Mcl-1) or induce (Bax, Bcl-x<sub>S</sub>, Bag and Bad) apoptosis in several systems. Bad was identified as a Bcl-2 interacting protein (1). It has homology to Bcl-2 within the Bcl-2 homolog domains 1 and 2 (BH1 and BH2). In mammalian cells, Bad selectively heterodimerizes with Bcl-x<sub>L</sub> as well as Bcl-2, but not with other Bcl-2 family members (Bax, Bcl-x<sub>S</sub>, Mcl-1 and A1). When Bad forms heterodimers with Bcl-x<sub>L</sub>, it displaces Bax from Bcl-x<sub>L</sub> and promotes cell death (1, 2).

This antibody reacts with the 30 kDa mouse and the 25 kDa human Bad, respectively, by immunoblotting, using total cell lysate from mouse NIH3T3, WR19L12a and human A431, HL60 cell lines.

**REAGENT**

Anti-Bad Unconjugated Polyclonal Antibody  
PN IM3526 – 0.2 mg – Liquid 0.1 mL

**CLONE**

Polyclonal

**IMMUNOGEN**

GST-mouse Bad fusion protein

**SPECIES**

Rabbit

**SOURCE**

Serum

**PURIFICATION**

Protein-A Sepharose

**POTENTIAL APPLICATIONS**

Immunoblotting.

**BUFFER**

This antibody is provided in phosphate-buffered saline (PBS), containing 50% glycerol.

**STORAGE CONDITIONS AND STABILITY**

This antibody is provided in liquid form and may be stored at -20°C until the expiration date stated on the vial label.

No preservative has been added.

**REAGENT PREPARATION**

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial.

**SUGGESTED PROCEDURE**

SDS PAGE & Immunoblotting

Working dilution 1:1000 for chemiluminescence detection.

- 1) Wash the cells 3 times with PBS and resuspend with 2 volumes of cold Prep buffer (Prep buffer: 50 mM Hepes pH 7.3, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate at 4°C with agitation for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g at 4°C for 10 minutes and transfer the supernatant to another tube. Measure the protein concentration and adjust to 8 mg/mL with Prep buffer.
- 3) Boil cell lysates or tissue homogenates in Laemli buffer (5 – 20 µg total protein) for 3 – 5 minutes and centrifuge at 12,000 x g for 1 minute. To each well of an SDS-polyacrylamide gel, load 10 µL of sample supernatant and perform electrophoresis in a 1 mm thick gel.
- 4) Transfer to polyvinylidene difluoride (PVDF) membrane at 10 V for 1 hour in a semi-dry transfer system (transfer buffer: 25 mM Tris, 190 mM glycine, 20% MeOH).
- 5) The transferred proteins can be visualized by staining the membrane for 1 minute with 0.1% Ponceau S (Sigma, Cat. No. P 7170) in 5% acetic acid. Rinse the membrane with PBS.
- 6) Non-specific binding sites are blocked by immersing the membrane in 5% skim milk / PBS / 0.05% Tween 20 for 1 hour at room temperature (18 – 25°C), or overnight at 4°C.
- 7) Incubate with primary antibody at the recommended dilution (the concentration of antibody to be used may vary depending on the studied tissue) for 1 hour at room temperature.
- 8) Wash the membrane 3 times for 5 – 10 minutes per wash with PBS / 0.05% Tween 20.
- 9) Incubate with Horseradish Peroxidase-conjugated goat anti-rabbit (PN IM0831) diluted in PBS / 0.05% Tween 20 for 45 minutes at room temperature.

3526EX050700 Vers. 01/ 07/07/00 AC-00-0741

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- 10) Wash the membrane 3 times for 10 minutes per wash with PBS / 0.05% Tween 20.
- 11) Incubate with Amersham ECL Reagent for 1 minute. Drain membrane, remove excess ECL Reagent by dabbing with a Kimwipe, and seal in plastic wrap.
- 12) Expose to ECL hyperfilm in a dark room for 30 seconds. Develop as usual for autoradiogram or X-ray. The conditions for development and exposure may have to be adapted.

Positive control: myc-tag-Bad expressed cell lysate (Code: 591-P, lyophilized form).

Cell lysate is available as a customer convenience to be used as a positive control for Western blotting. The myc-tag-Bad overexpressed cell lysate was derived from pCMV-myc-tag-Bad transfected 293T cell line.

**STATEMENT OF WARNINGS**

1. Specimens, samples and all material coming in contact with them should be handled as if capable

- of transmitting infection and disposed of with proper precautions.
2. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
3. Do not use antibody beyond the expiration date on the label.
4. Do not expose reagents to strong light during storage or incubation.
5. Avoid microbial contamination of reagents or incorrect results might occur.

**SELECTED RESEARCH REFERENCES**

1. [324] Yang, E., Zha, J., Jockel, J., Boise, L.H. Thompson, C.B., Korsmeyer, S.J., "Bad, a heterodimeric partner for Bcl-x<sub>L</sub> and Bcl-2, displaces Bax and promotes cell death", 1995, Cell, 2, 80, 285-291.
2. [325] Gauthier, E.R., Piché, L., Lemieux, G., Lemieux, R., "Role of bcl-x<sub>L</sub> in the control of apoptosis in murine myeloma cells", 1996, Cancer Res., 6, 56, 1451-1456.