

RABBIT ANTI-BIM_{EL} (pS69) HUMAN / (pS65) RAT PHOSPHOSPECIFIC POLYCLONAL ANTIBODY

CATALOG NUMBER: AB3579

QUANTITY: 100 µL

LOT:

BACKGROUND:

Bim (bcl-2-interacting mediator of cell death) is a proapoptotic member of the Bcl-2 family that shares only the BH3 domain with this family. There are three isoforms of Bim: Bim_{EL}, Bim_L, and Bim_S. Bim is involved in regulating the intrinsic mitochondrial apoptotic pathway by inducing cytochrome c release, which in turn, activates caspase-9 and then caspase-3. Bim also plays a critical role in central and peripheral deletion of T lymphocytes and in controlling B cell homeostasis and activation. Bim_{EL}, the long isoform of Bim, is a ~28 kDa protein that is predominantly expressed in T and B cells and is activated by ERK1/2 pathway. The activation of Bim_{EL} by ERK1/2 promotes its phosphorylation on serine 65 (serine 69 in human), targeting it for degradation via the proteasome.

IMMUNOGEN:

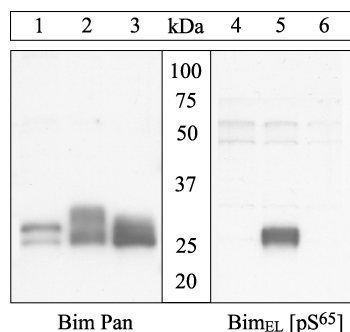
The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of rat Bim_{EL} that contains serine 65 (serine 69 in the human sequence). The sequence is conserved in human and mouse.

APPLICATIONS:

For Western blotting applications, we recommend using the antibody at a 1:1000 dilution.

Western Blotting Experiment

Lysates prepared from Hek293T cells transfected with rat WT Bim_{EL} alone (1, 4), cotransfected with rat WT Bim_{EL} and activated MEKK1 (MEKK1Δ) (2, 5) or with mutant S65A Bim_{EL} and MEKK1Δ (3, 6), were resolved by SDS-PAGE on a 14% polyacrylamide gel and transferred to PVDF. Membranes were blocked with 3% Milk-TBST buffer for one hour at room temperature, and incubated with Bim Pan antibody (1, 2, 3) or Bim_{EL} [pS⁶⁵] (4, 5, 6) for two hours at room temperature in 3% Milk-TBST buffer. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG HRP conjugate and bands were detected using standard methods. The data show that the signal is detected only in lysates co-expressing rat WT Bim_{EL} and active MEKK1 (MEKK1Δ). This signal is abolished in cells expressing mutant rat Bim_{EL} S65A and MEKK1, verifying that the signal is site-phosphospecific.



Optimal working dilutions must be determined by end user.

SPECIES REACTIVITY:

Rat Bim_{EL}. This antibody is observed to react with human Bim_{EL} when phosphorylated at serine 69. Mouse Bim_{EL} (100% homologous) has not been tested, but is expected to react.

CONTROL:

Hek293T co-expressing rat Bim_{EL} and active MEKK1 (MEKK1Δ).

FORMAT:

Purified from rabbit serum by sequential epitope-specific chromatography. The antibody



has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated Bim. The final product is generated by affinity chromatography using a Bim_{EL}-derived peptide that is phosphorylated at serine 65 (serine 69 in the human sequence).

- PRESENTATION:** Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier. 0.05% sodium azide
- STORAGE/HANDLING:** Store at -20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at -20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
- REFERENCES:**
- Yip, K.W., et al. (2004) Potential utility of bim(s) as a novel apoptotic therapeutic molecule. *Mol. Ther.* 10(3):533-544.
- Ley, R. et al. (2004) Extracellular signal-regulated kinases 1/2 are serum-stimulated "Bim(EL) kinases" that bind to the BH3-only protein Bim(EL) causing its phosphorylation and turnover. *J. Biol. Chem.* 279(10):8837-8847.
- Chen, D, and Q. Zhou (2004) Caspase cleavage of BimEL triggers a positive feedback amplification of apoptotic signaling. *Proc. Nat'l. Acad. Sci.* 101(5):1235-1240.
- Jiang, Z. et al. (2004) Lovastatin-induced up-regulation of the BH3-only protein, Bim, and cell death in glioblastoma cells. *J. Neurochem.* 89(1):168-178.
- Mouhamad, S. et al. (2004) B cell receptor-mediated apoptosis of human lymphocytes is associated with a new regulatory pathway of Bim isoform expression. *J. Immunol.* 172(4):2084-2091.
- Luciano, F. et al. (2003) Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the proteasome pathway and regulates its proapoptotic function. *Oncogene* 22(43):6785-6793.
- O'Connor, L. et al. (1998) Bim: a novel member of the Bcl-2 family that promotes apoptosis. *The EMBO J.* 17:384-395.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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