BP7010

OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Polyclonal Antibody to BimEL pSer65 - Aff - Purified

Alternate names: Apoptosis Facilitator, BAM, BCL2L11, BOD, Bcl-2-like Protein 11, Bcl2-interacting Mediator of Cell Death, BimEL, BimL, BimS

Catalog No.: BP7010

Quantity: 0.1 ml

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: BimEL, BimL, and BimS. 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. BimEL, 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 BimEL by ERK1/2 promotes its phosphorylation on serine 65 (serine 69 in human), targeting it for degradation via the proteasome.

Host: Rabbit

Immunogen: A chemically synthesized phosphopeptide derived from the region of rat BimEL that contains serine 65 (serine 69 in the human sequence).
Remarks: The sequence is conserved in human and mouse.

Format: **State:** Liquid Ig fraction
Purification: 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 BimEL-derived peptide that is phosphorylated at serine 65 (serine 69 in the human sequence).
Buffer System: Dulbeccos 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 and 0.05% sodium azide as preservative.

Applications: Western blot: use at a 1:1000 starting dilution.
Positive control used: Hek293T co-expressing rat BimEL and active MEKK1 (MEKK1delta); background extract +/- recombinant Bim-GST activated with JNK1 (3.5 µg/per 1 µg protein, 40 minutes, 37°C)
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The antibody reacts with rat BimEL. It is observed to react with human BimEL when phosphorylated at serine 69. Mouse BimEL (100% homologous) has not been tested, but is expected to react.

Storage:

Can be shipped at 2-8°C. Upon arrival, store in aliquots at -20°C. Avoid repeated freezing and thawing.
(We recommend a brief centrifugation before opening to settle vial contents).
Shelf life: one year from despatch.

General Readings:

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.
OConnor, L. et al. (1998) Bim: a novel member of the Bcl-2 family that promotes apoptosis. The EMBO J.17:384-395.

Protocols:

Western Blotting Procedure:
1. Lyse approximately 10e7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use . Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifugedat 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100oC.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the PVDF membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4oC.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered

saline supplemented with 3% non-fat dried milk and 0.1% Tween 20 for one hour at room temperature or overnight at 4oC.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab)2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.
Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add 1 mL 10% SDS.
Cool to 4oC prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
3 gm non-fat dried milk
0.1 mL Tween 20

Pictures:

Western Blotting Experiment: Lysates prepared from Hek293T cells transfected with rat WT BimEL alone (1, 4), cotransfected with rat WT BimEL and activated MEKK1 (MEKK1Δ) (2, 5) or with mutant S65A BimEL 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 BimEL [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 the Pierce SuperSignal(TM) method. The data show that the signal is detected only in lysates co-expressing rat WT BimEL and active MEKK1 (MEKK1Δ).This signal is abolished in cells expressing mutant rat BimEL S65A and MEKK1, verifying that the signal is site-phosphospecific.