Rabbit (polyclonal) Anti-Bim_{EL} [pS^{69}] (Human) / [pS^{65}] Rat Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44431G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	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).
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.
Target Summary:	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.
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.
Applications:	The antibody has been used in Western blotting. Other applications may work but have not been tested.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	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.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Controls:	Hek293T co-expressing rat Bim_{EL} and active MEKK1 (MEKK1Δ); background extract +/- recombinant Bim-GST activated with JNK1 (3.5 µg per 1 µg protein, 40 minutes, 37°C).

This product is for research use only. Not for use in diagnostic procedures.

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Related Products:

Antibodies:

BAD [pS[128]], Cat. # 44523G

Bcl-XL [pS[62]], Cat. # 44428G

BID [59/60] CSSA, Cat. # 44436G

BID [p15] CSSA, Cat. # 44433G

NFκB [pS[529]], Cat. # 44711G

HSP27 [pS[82]], Cat. # 44534G

ERK1&2 [pTpY[185/187]], Cat. # 44680G

JNK [pTpY183/185], Cat. # 44682G

ELISA:

Bcl-2 [pS[70]] Elisa Kit, Cat. # KHO0311

References:

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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.

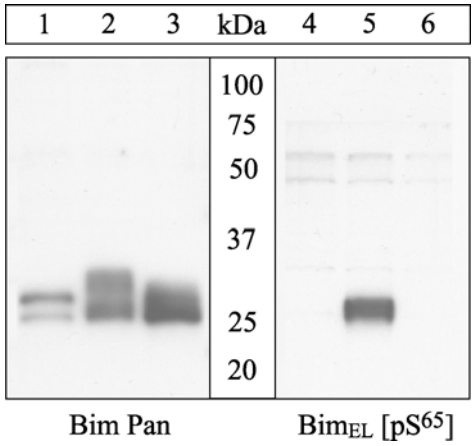
Chen, D, and Q. Zhou (2004) Caspase cleavage of BimEL triggers a positive feedback amplification of apoptotic signaling. Proc. Nat'l. Acad. Sci. 101(5):1235-1240.

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.



Western Blot Mutant Analysis

Extracts of Hek293T cells transfected with rat wild type Bim_{EL} alone (1, 4), co-transfected with rat wild type 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% Tris-glycine gel and transferred to PVDF. The membrane was blocked with 3% milk-TBST buffer for one hour at room temperature and then incubated with a Bim pan antibody (1-3) or the Bim_{EL} [pS[65]] antibody (4-6) for two hours at room temperature in 3% milk-TBST buffer. After washing, the membrane was incubated with goat $F(ab')_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that the phosphosignal is detected only when rat wild type Bim_{EL} and active MEKK1 (MEKK1Δ) are co-expressed. This signal is abolished in cells expressing mutant rat Bim_{EL} S65A and MEKK1, verifying that the signal is phosphorylation site-specific.

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Western Blotting Procedure

1. Lyse approximately 10^7 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 (Invitrogen cat. # FNN0011). 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 ultracentrifuged at 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 100°C.
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 ddH_2O 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 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% non-fat dried milk and 0.1% Tween 20 for two hours at room temperature or overnight at 4°C.
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 (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) 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 $Na_4P_2O_7$
2 mM Na_3VO_4
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 4°C 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

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