BP7006

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Polyclonal Antibody to BCL2L1 / Bcl-XL pSer62 - Aff - Purified

Alternate names: Apoptosis Regulator Bcl-X, BCL2L, BCL2L1, BCLX, BCLXL, Bcl-2-like 1 Protein, bcl-xL

Catalog No.: BP7006

Quantity: 0.1 ml

Background: Bcl-xL is a ~28 kDa member of Bcl-2 family of proteins and an important regulator of apoptosis. Bcl-xL forms heterodimers with BAX, BAK, and Bcl-2, and its overexpression in tumor cells confers resistance against chemotherapeutic drugs. Bcl-xL is phosphorylated on many sites including serine 62, a critical site for Bcl-xL response to microtubule-damaging drugs such as taxol and vinblastine. Phosphorylation of serine 62 - thought to be mediated by Jun N-terminal stress kinase (JNK) signaling - negatively regulates the anti-apoptotic function of Bcl-xL and controls the growth of neoplastic cells.

Host: Rabbit

Immunogen: Chemically synthesized phosphopeptide derived from the region of human Bcl-xL that contains serine 62.
Remarks: The sequence is conserved in mouse and rat.

Format: **State:** Liquid Ig fraction
Purification: Epitope affinity 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 Bcl-xL. The final product is generated by affinity chromatography using a Bcl-xL-derived peptide that is phosphorylated at serine 62.
Buffer System: 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 as preservative

Applications: Western blot (1:1,000).
Use SK-BR-3 cells treated with vinblastine as positive control.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody detects Bcl-xL.
Species: Human, mouse and rat.
Other species not tested.

Storage: Store at 2 - 8 °C up to one week or (in aliquots) at -20 °C for longer. Centrifuge vial before opening. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: Mc Gee, M.M., et al. (2004) Selective induction of apoptosis by PBOX-6 in leukemia cells occurs via the JNK dependent phosphorylation and inactivation of Bcl-2 and Bcl-XL. J.

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Raina, D., et al. (2004) The MUC1 oncoprotein activates the anti-apoptotic phosphoinositide 3-kinase/Akt and Bcl-xL pathways in rat 3Y1 fibroblasts. J. Biol. Chem. 279(20):20607-20612.
Assaf, H., et al. (2004) Ochratoxin A induces apoptosis in human lymphocytes through downregulation of Bcl-xL. Toxicol. Sci. 79:335-344.
Wang, S., et al. (2003) Targeting Bcl-2 and Bcl-XL with nonpeptidic small-molecule antagonists. Semin. Oncol. 30(5 Suppl 16):133-142. Review.
Basu, A. and S. Haldar (2003) Identification of a novel Bcl-xL phosphorylation site regulating the sensitivity of taxol- or 2-methoxyestradiol-induced apoptosis. FEBS Lett. 538(1-3):41-47.
Sugiyama, K., et al. (1999) Decrease in susceptibility toward induction of apoptosis and alteration in G1 checkpoint function as determinants of resistance of human lung cancer cells against the antisignaling drug UCN-01 (7-Hydroxystaurosporine). Cancer Res. 59(17):4406-4412.

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. 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 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 ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the 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 dilution in Tris buffered saline supplemented with 3% BSA and 0.1% Tween 20 overnight at 4°C or for one hour at room temperature.
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 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
5 gm BSA
0.1 mL Tween 20

Pictures:

Peptide Competition Lysates prepared from SK-BR3 cells left untreated (1) or treated with vinblastine (2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer for one hour at room temperature and incubated with Bcl-xL [pS62] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphoserine-containing peptide (4), or, the phosphopeptide immunogen (5). 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 only the peptide corresponding to Bcl-xL [pS62] blocks the signal, verifying the specificity of the antibody.