# Rabbit (polyclonal) Anti-Bcl-xL [pS$^{62}$] Phosphospecific Antibody, Unconjugated

## PRODUCT ANALYSIS SHEET

| | |
|---|---|
| **Catalog Number:** | 44428G (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 Bcl-xL. The final product is generated by affinity chromatography using a Bcl-xL-derived peptide that is phosphorylated at serine 62. |
| **Immunogen:** | The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human Bcl-xL that contains serine 62. The sequence is conserved in mouse and rat. |
| **Target Summary:** | 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. |
| **Reactivity:** | Human Bcl-xL. Mouse and rat Bcl-xL (100% homologous) have not been tested, but are expected to react. |
| **Applications:** | The antibody has been used in Western blotting applications. 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 exact concentration is not determined for each lot; however, the typical range is 0.1-1.0 mg/mL. 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 Control Used:** | SK-BR-3 cells treated with vinblastine. |



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This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

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**Related Products:**

**Antibodies:**

| | |
|---|---|
| STAT5 [pY$^{694}$], Cat. # 44390G | BAD [pS$^{128}$], Cat. # 44523G |
| SHP2 [pY$^{542}$], Cat. # 44554G | Akt/PKB, Cat. # 44609G |
| SHP2 [pY$^{580}$], Cat. # 44558G | Akt/PKB [pT$^{308}$], Cat. # 44602G |
| JNK [pTpY$^{183/185}$], Cat. # 44682G | Akt/PKB [pS$^{473}$], Cat. # 44623G |

**References:**


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Jin, Y.P., et al. (2004) Anti-HLA class I antibody-mediated activation of the PI3K/Akt signaling pathway and induction of Bcl-2 and Bcl-xL expression in endothelial cells. Hum. Immunol. 65(4):291-302.

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.

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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.

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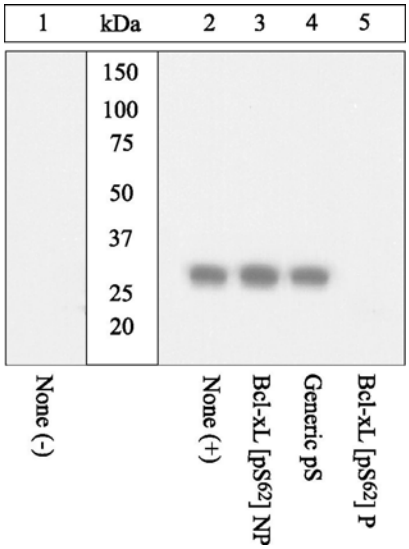


**Up-regulation and Antibody-Peptide Competition**

Extracts of SK-BR-3 cells left untreated (1) or treated with 30 nM vinblastine for one hour (2-5) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, then incubated with the Bcl-xL [pS$^{62}$] 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 phosphopeptide immunogen (3), a generic phosphoserine-containing peptide (4), or the phosphopeptide immunogen (5). 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 only the phosphopeptide corresponding to Bcl-xL [pS$^{62}$] blocks the signal, demonstrating the specificity of the antibody. The data also show up-regulation upon vinblastine treatment in this cell system.




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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) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 for overnight at 4°C or for two hours 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 (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
5 gm Ig-free BSA
0.1 mL Tween 20



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