Store at –20°C

#9941

Pro-Survival Bcl-2 Family Antibody Sampler Kit

☑ 1 Kit
(5 x 40 µl)

Cell Signaling TECHNOLOGY®

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Products Included	Product #	Quantity	Mol. Wt.	Source
Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb	2827	40 µl	28 kDa	Rabbit IgG
Phospho-Bcl-2 (Thr56) Antibody (Human Specific)	2875	40 µl	28 kDa	Rabbit IgG
Bcl-2 (50E3) Rabbit mAb	2870	40 µl	26 kDa	Rabbit IgG
Bcl-xL (54H6) Rabbit mAb	2764	40 µl	30 kDa	Rabbit IgG
Mcl-1 (D35A5) Rabbit mAb	5453	40 µl	40, 35 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Pro-Survival Bcl-2 Family Antibody Sampler Kit provides an economical means to examine several members of the Bcl-2 family. The kit contains enough primary and secondary antibodies to perform four western blot experiments.

Background: The Bcl-2 family consists of a number of evolutionarily conserved proteins containing Bcl-2 homology domains (BH) that regulate apoptosis through control of mitochondrial membrane permeability and release of cytochrome c (1-3). Four BH domains have been identified (BH1-4), which mediate protein interactions. The family can be separated into three groups based upon function and sequence homology: pro-surivival members including Bcl-2, Bcl-xL, Mcl-1, A1 and Bcl-w; pro-apoptotic proteins including Bax, Bak and Bok, and "BH3 only" proteins Bad, Bik, Bid, Puma, Bim, Bmf, Noxa and Hrk. Interactions between death-promoting and death-suppressing Bcl-2 family members has led to a rheostat model in which the ratio of pro-apoptotic and anti-apoptotic proteins controls cell fate (4). Thus, pro-survival members exert their behavior by binding to and antagonizing death-promoting members. In general, the "BH3-only members" can bind to and antagonize the pro-survival proteins leading to increased apoptosis (5). While some redundancy of this system likely exists, tissue specificity, transcriptional and post-translational regulation of many of these family members can account for distinct physiological roles.

Several phosphorylation sites have been identified within Bcl-2 including Thr56, Ser70, Thr74 and Ser87 (6). These phosphorylation sites may be targets of the ASK1/MKK7/JNK1 pathway, and phosphorylation of Bcl-2 may be a marker for mitotic events (7,8). Mutation of Bcl-2 at Thr56 or Ser87 inhibits its anti-apoptotic activity during glucocorticoid-induced apoptosis of T lymphocytes (9). Interleukin 3 and JNK-induced Bcl-2 phosphorylation at Ser70 may be required for its enhanced antiapoptotic functions (10).

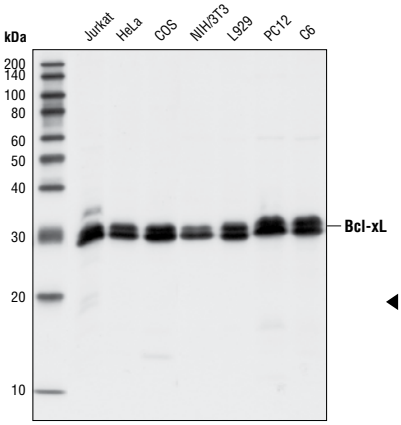
Specificity/Sensitivity: Each antibody in the Pro-Survival Bcl-2 Family Antibody Sampler Kit recognizes only its specific target. The antibodies do not cross-react with other Bcl-2 family members. Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb detects endogenous levels of human Bcl-2 only when phosphorylated at Ser70. Phospho-Bcl-2 (Thr56) Antibody (Human Specific) detects endogenous levels of human Bcl-2 only when phosphorylated at Thr56.

Source/Purification: Total Mcl-1, Bcl-xL, and Bcl-2 monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Leu210 of human Mcl-1, Asp61 of human Bcl-xL, and residues at the carboxy terminus of human Bcl-2 alpha. Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser70 of human Bcl-2. Phospho-Bcl-2 (Thr56) Antibody (Human Specific) is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr56 of human Bcl-2. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot the antibody.*

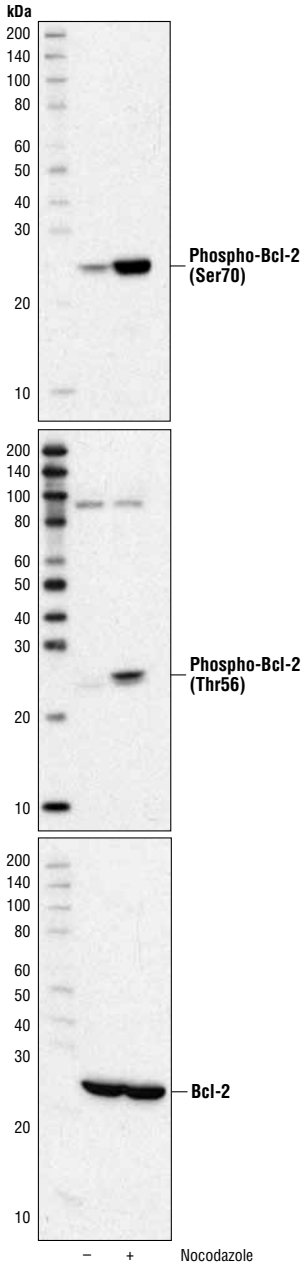
Recommended Antibody Dilutions:
Western blotting 1:1000
See www.cellsignal.com for individual component dilutions and additional application protocols.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

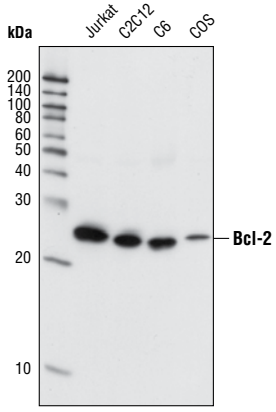


◀ *Western blot analysis of extracts from Jurkat and HeLa (human), COS (monkey), NIH/3T3 and L929 (mouse), and PC12 and C6 (rat) cells, using* ***Bcl-xL (54H6) Rabbit mAb #2764.***

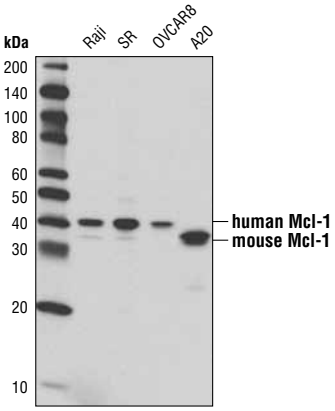
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of extracts from THP-1 cells, untreated or nocadazole-treated (1.0 µg/mL) overnight, using ***Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb #2827*** *(upper),* ***Phospho-Bcl-2 (Thr56) Antibody (Human Specific) #2875*** *(middle) and* ***Bcl-2 (50E3) Rabbit mAb #2870*** *(lower).*



Western blot analysis of extracts from various cell types using ***Bcl-2 (50E3) Rabbit mAb #2870.***



Western blot analysis of extracts from various cell lines using ***Mcl-1 (D35A5) Rabbit mAb #5453****.*

Background References:

(1) Cory, S. et al. (2003) *Oncogene* 22, 8590–8607.

(2) Antonsson, B. and Martinou, J. (2000) *Exp. Cell Res.* 256, 50–57.

(3) Sharpe, J.C. et al. (2004) *Biochim. Biophys. Acta.* 1644, 107–113.

(4) Korsmeyer, S.J. et al. (1993) *Semin. Cancer Biol.* 4, 327–337.

(5) Bouillet, P. and Strasser, A. (2002) *J. Cell Sci.* 115, 1567–1574.

(6) Maundrell, K. et al. (1997) *J. Biol. Chem.* 272, 25238–25242.

(7) Yamamoto, K. et al. (1999) *Mol. Cell Biol.* 19, 8469–8478.

(8) Ling, Y. H. et al. (1998) *J. Biol. Chem.* 273, 18984–18991.

(9) Huang, S.J. and Cidlowski, J.A. (2002) *FASEB* 16, 825–832.

(10) Deng, X. et al. (2001) *J. Biol. Chem.* 276, 23681–23688.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.