Store at -20°C

#17229

Pro-Survival Bcl-2 Family Antibody Sampler Kit II

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Entrez-Gene ID #597, #599, #596, #598, #4170
UniProt ID #Q16548, #Q92843, #P10415, #Q07817

New 10/17

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Bcl-2 (D55G8) Rabbit mAb (Human Specific)	4223	20 µl	26 kDa	Rabbit IgG
Phospho-Bcl-2 (S70) (5H2) Rabbit mAb	2827	20 µl	28 kDa	Rabbit IgG
Mcl-1 (D2W9E) Rabbit mAb	94296	20 µl	40 (human) kDa 35 (mouse) kDa	Rabbit IgG
Phospho-Mcl-1 (Thr163) (D5M9D) Rabbit mAb	14765	20 µl	40 kDa	Rabbit IgG
Bcl-xL (54H6) Rabbit mAb	2764	20 µl	30 kDa	Rabbit IgG
A1/Bfl-1 (D1A1C) Rabbit mAb	14093	20 µl	18 kDa	Rabbit IgG
Bcl-w (31H4) Rabbit mAb	2724	20 µl	18 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 II provides an economical means to examine several members of the Bcl-2 family. The kit contains enough primary antibody to perform two 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) that mediate protein interactions. The family can be separated into three groups based upon function and sequence homology: pro-survival members include Bcl-2, Bcl-xL, Mcl-1, A1 and Bcl-w; pro-apoptotic proteins include Bax, Bak and Bok, and "BH3" 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 anti-apoptotic functions (10). Mcl-1 is phosphorylated in response to treatment with phorbol ester, microtubule-damaging agents, oxidative stress, and cytokine withdrawal (11-14). Phosphorylation at Thr163, the conserved MAP kinase/ERK site located within the PEST region, slows Mcl-1 protein turnover (13) but may prime the GSK-3 mediated phosphorylation at Ser159 that leads to Mcl-1 destabilization (14).

Specificity/Sensitivity: Each antibody in the Pro-Survival Bcl-2 Family Antibody Sampler Kit II recognizes endogenous levels of its specific target. The antibodies do not cross-react with other Bcl-2 family members. A1/Bfl-1 (D1A1C) Rabbit mAb may cross-react with an unknown protein at 50 and 130 kDa in some cell lines. Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb detects endogenous of human Bcl-2 only when phosphorylated at Ser70. Phospho-Mcl-1 (Thr163) (D5M9D) Rabbit mAb recognizes endogenous levels of Mcl-1 only when phosphorylated at Thr163. This antibody may also cross-react with an unidentified protein at 70 kDa in some cell lines.

Source/Purification: Rabbit monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Gly47 of human Bcl-2, Asp61 of human Bcl-xL, Pro60 of mouse Mcl-1, Gly29 of human A1/Bfl-1, and Ala39 of human Bcl-w. Phospho-specific rabbit monoclonal antibodies are produced by immunizing animals with synthetic phospho-peptides corresponding to residues surrounding Ser70 of human Bcl-2 and Thr163 of human Mcl-1.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Background References:

(1) Cory, S. et al. (2003) *Oncogene* 22, 8590-607.
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(3) Sharpe, J.C. et al. (2004) *Biochim Biophys Acta* 1644, 107-13.
(4) Korsmeyer, S.J. et al. (1993) *Semin Cancer Biol* 4, 327-32.
(5) Bouillet, P. and Strasser, A. (2002) *J Cell Sci* 115, 1567-74.
(6) Maundrell, K. et al. (1997) *J Biol Chem* 272, 25238-42.
(7) Yamamoto, K. et al. (1999) *Mol Cell Biol* 19, 8469-78.
(8) Ling, Y.H. et al. (1998) *J Biol Chem* 273, 18984-91.
(9) Huang, S.T. and Cidlowski, J.A. (2002) *FASEB J* 16, 825-32.
(10) Deng, X. et al. (2001) *J Biol Chem* 276, 23681-88.
(11) Domina, A.M. et al. (2000) *J Biol Chem* 275, 21688-94.
(12) Inoshita, S. et al. (2002) *J Biol Chem* 277, 43730-34.
(13) Domina, A.M. et al. (2004) *Oncogene* 23, 5301-15.
(14) Maurer, U. et al. (2006) *Mol Cell* 21, 749-60.

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Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity: 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 Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.