

Store at
-20°C

#98322

Pro-Apoptosis Bcl-2 Family Antibody Sampler Kit II



Support: +1-978-867-2388 (U.S.)
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New 11/17

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
Bax (D2E11) Rabbit mAb	5023	20 µl	20 kDa	Rabbit IgG
Bak (D4E4) Rabbit mAb	12105	20 µl	25 kDa	Rabbit IgG
Bok (D7V2N) Rabbit mAb	86875	20 µl	22 kDa	Rabbit IgG
Bim (C34C5) Rabbit mAb	2933	20 µl	12, 15, 23 kDa	Rabbit IgG
Bad (D24A9) Rabbit mAb	9239	20 µl	23 kDa	Rabbit IgG
BID (Human Specific) Rabbit Antibody	2002	20 µl	15, 22 kDa	Rabbit
Puma (D30C10) Rabbit mAb	12450	20 µl	23 kDa	Rabbit IgG
Noxa (D8L7U) Rabbit mAb	14766	20 µl	10 kDa	Rabbit IgG
Bik Rabbit Antibody	4592	20 µl	20 kDa	Rabbit
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-Apoptosis 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 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.

Background References:

- (1) Cory, S. et al. (2003) *Oncogene* 22, 8590-607.
- (2) Antonsson, B. and Martinou, J.C. (2000) *Exp Cell Res* 256, 50-7.
- (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.

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 antibodies.*

Recommended Antibody Dilutions:

Western blotting 1:1000

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

Specificity/Sensitivity: Each antibody in the Pro-Apoptosis Bcl-2 Family Antibody Sampler Kit II recognizes endogenous levels of its specific target. Bim (C34C5) Rabbit mAb detects endogenous levels of total Bim (EL, L, and S isoforms) protein. BID Antibody (Human Specific) detects endogenous levels of both the full length (22 kDa) and cleaved large fragment (15 kDa) of human BID. Puma (D30C10) Rabbit mAb cross-reacts with a protein of unknown origin at 60 kDa. Noxa (D8L7U) Rabbit mAb cross-reacts with multiple unidentified proteins, most notably at 35, 50, and 80 kDa.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Leu45 of human Bax, Gly75 of human Bak, Val88 of human Bok, Pro25 of human Bim, Pro102 of human Bad, Leu45 of human Bax, Pro25 of human Bim, residues near the carboxy terminus of human Puma, and residues near the amino terminus of human Noxa. Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to the amino-terminus of human Bik or residues surrounding the cleavage site of human BID. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

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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 dH₂O, mix.
- 2. 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, 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 dH₂O.
- 4. 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, 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 dH₂O, 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 dH₂O, 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:** (#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 cm²) 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.

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