

# Pro-Apoptosis Bcl-2 Family Antibody Sampler Kit

✓ 1 Kit  
(8 x 20 µl)



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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Bad (D24A9) Rabbit mAb	9239	20 µl	23 kDa	Rabbit IgG
Phospho-Bad (Ser112) (40A9) Rabbit mAb	5284	20 µl	23 kDa	Rabbit IgG
Bax (D2E11) Rabbit mAb	5023	20 µl	20 kDa	Rabbit IgG
Bik Antibody	4592	20 µl	20 kDa	Rabbit IgG
Bim (C34C5) Rabbit mAb	2933	20 µl	12,15,23 kDa	Rabbit IgG
BID Antibody (Human Specific)	2002	20 µl	15, 22 kDa	Rabbit IgG
Bak (D4E4) Rabbit mAb	12105	20 µl	25 kDa	Rabbit IgG
Puma (D30C10) Rabbit mAb	12450	20 µl	23 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://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 provides an economical means to examine several members of the Bcl-2 family and their activation status. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**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-survival 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.

Bad is a pro-apoptotic member of the Bcl-2 family that can

displace Bax from binding to Bcl-2 and Bcl-xL, resulting in cell death (6,7). Survival factors such as IL-3 can inhibit the apoptotic activity of Bad by activating intracellular signaling pathways that result in the phosphorylation of Bad at Ser112 and Ser136 (7). Phosphorylation at these sites results in the binding of Bad to 14-3-3 proteins and the inhibition of Bad binding to Bcl-2 and Bcl-xL (7). Akt has been shown to promote cell survival via its ability to phosphorylate Bad at Ser136 (8,9). Ser112 has been shown to be the substrate *in vivo* and *in vitro* of p90RSK (10,11) and mitochondria-anchored PKA (12).

**Specificity/Sensitivity:** Each antibody in the Pro-Apoptosis Bcl-2 Family Antibody Sampler Kit recognizes only its specific target. The antibodies do not cross-react with other Bcl-2 family members. Phospho-Bad (Ser112) (40A9) Rabbit mAb detects endogenous levels of Bad only when phosphorylated at Ser112 (mouse), Ser75 (human), or Ser113 (rat). Puma (D30C10) Rabbit mAb recognizes endogenous levels of total Puma protein but also cross-reacts with a protein of unknown origin at 60 kDa.

**Source/Purification:** Phospho-Bad (Ser112) (40A9) Rabbit mAb is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser112 of mouse Bad. Total Bad, Bax, Bim, Bak and Puma monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Pro102 of human Bad, Leu45 of human Bax, Pro25 of human Bim, Gly75 of human Bak, or near the

**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 antibodies.*

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

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carboxy terminus of human Puma. 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.

**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) Yang, E. et al. (1995) *Cell* 80, 285–291.
- (7) Zha, J. et al. (1996) *Cell* 87, 619–628.
- (8) Datta, S. R. et al. (1997) *Cell* 91, 231–241.
- (9) Peso, L. et al. (1997) *Science* 278, 687–689.
- (10) Bonni, A. et al. (1999) *Science* 286, 1358–1362.
- (11) Tan, Y. et al. (1999) *J. Bio. Chem.* 274, 34859–34867.
- (12) Harada, H. et al. (1999) *Mol. Cell* 3, 413–422.

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# 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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH<sub>2</sub>O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

## B. Protein Blotting

**A general protocol for sample preparation.**

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

## C. Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

### I. Membrane Blocking

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

### II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

## D. Detection of Proteins

- 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.
- 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.