

IAP Family Antibody Sampler Kit



✓ 1 Kit
 (5 x 20 µl)

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
c-IAP1 (D5G9) Rabbit mAb	7065	20 µl	62 kDa	Rabbit IgG
c-IAP2 (58C7) Rabbit mAb	3130	20 µl	70 kDa	Rabbit IgG
Survivin (71G4B7) Rabbit mAb	2808	20 µl	16 kDa	Rabbit IgG
XIAP (3B6) Rabbit mAb	2045	20 µl	53 kDa	Rabbit IgG
Livin (D61D1) XP® Rabbit mAb	5471	20 µl	34, 36 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 IAP Family Antibody Sampler Kit provides an economical means to investigate the expression of various IAP family members within the cell. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The inhibitor of apoptosis protein (IAP) family consists of an evolutionarily conserved group of apoptosis inhibitors containing a conserved 70 amino acid BIR (baculovirus inhibitor repeat) domain (1,2). Human members of this family include c-IAP1, c-IAP2, XIAP, survivin, livin, and NAIP. Overexpression of IAP family members, particularly survivin and livin, in cancer cell lines and primary tumors suggests an important role for these proteins in cancer progression (3-5). In general, the IAP proteins function through direct interactions to inhibit the activity of several caspases, including caspase-3, caspase-7, and caspase-9 (5,6). In addition, binding of IAP family members to the mitochondrial protein Smac blocks their interaction with caspase-9, thereby allowing the processing and activation of the caspase (2).

Specificity/Sensitivity: Each antibody in the IAP Family Antibody Sampler Kit detects endogenous levels of its respective target.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu28 of human c-IAP1 protein. The antibody is purified by protein A and affinity chromatography. Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Val117 of human c-IAP2, Cys60 of human Survivin, Ser245 of human XIAP, and Ala195 of human Livin.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 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.

Background References:

- (1) Deveraux, Q.L. and Reed, J.C. (1999) *Genes Dev* 13, 239-52.
- (2) Deveraux, Q.L. et al. (1998) *EMBO J* 17, 2215-23.
- (3) Altieri, D.C. et al. (1999) *Lab Invest* 79, 1327-33.
- (4) Tamm, I. et al. (2000) *Clin Cancer Res* 6, 1796-803.
- (5) Kasof, G.M. and Gomes, B.C. (2001) *J Biol Chem* 276, 3238-46.
- (6) Deveraux, Q.L. et al. (1997) *Nature* 388, 300-4.

U.S. Patent No. 5,675,063

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 All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- 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.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- 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 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

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.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- 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.

- 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.