

Inhibitor of Apoptosis Protein Detection Set

CATALOG No.: PSI-1815

BACKGROUND:

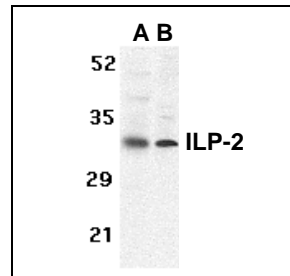
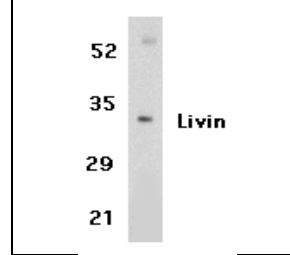
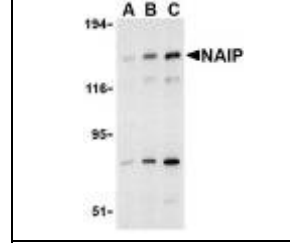
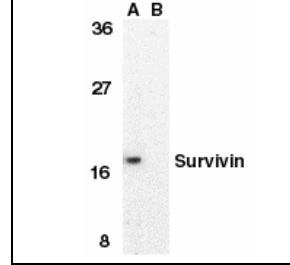
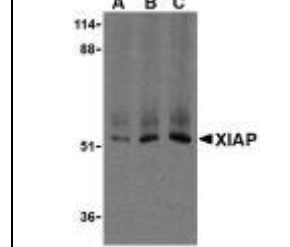
Apoptosis plays a major role in normal organism development, tissue homeostasis, and removal of damaged cells (reviewed in 1). This process can be interrupted by a family of proteins termed inhibitors of apoptosis (IAP). Members of the IAP family contain one to three copies of an approximately 70 amino acid motif termed baculovirus IAP repeat (BIR). These BIRs promote protein-protein interactions with members of the TRAF family of signaling molecules as well as caspases such as caspase-3, -7, and -9, and inhibiting the apoptotic activity of these proteins (reviewed in 2). Neuronal apoptosis inhibitor protein (NAIP) was the first human inhibitor of apoptosis protein (IAP) identified and was discovered by its association with the neurodegenerative disorder spinal muscular atrophy (3). Other IAPs include the X-linked protein XIAP/ILP-1 (4), the related cIAP protein which exists in two distinct isoforms (5), Livin (also known as KIAP as it is highly expressed in kidney) (6), Survivin/TIAP (7), ILP-2 (8), and Bruce, an extremely large IAP with ubiquitin-conjugating enzyme activity (9). Upregulation of these proteins inhibit the normal apoptotic process and thus may be involved in human diseases such as cancer.

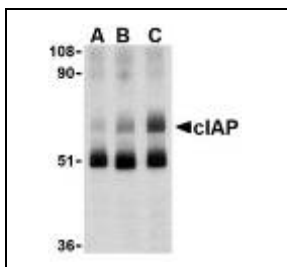
KIT CONTENTS:

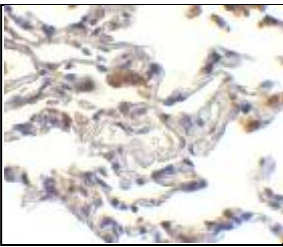
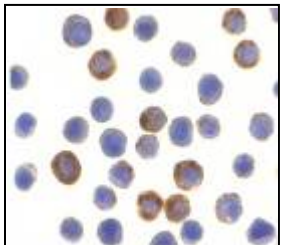
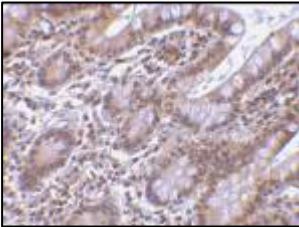
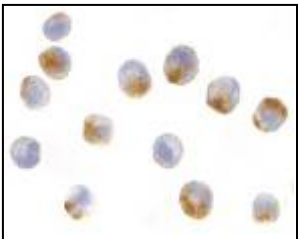

- clAP antibody, **Catalog No. 3325 (50µg)**.
- ILP-2 antibody, **Catalog No. 3017 (50µg)**.
- Livin antibody, **Catalog No. 2505 (50µg)**.
- NAIP antibody, **Catalog No. 3315 (50µg)**.
- Survivin antibody (CT), **Catalog No. 2235 (50µg)**.
- XIAP antibody, **Catalog No. 3331 (50µg)**.

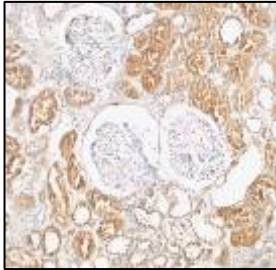
SOURCE:

Rabbit polyclonal antibodies were raised against peptides corresponding to amino acid sequences from each of the corresponding proteins.

	Western blot analysis of ILP-2 in (A) HepG2 cell lysate and (B) MOLT cell lysate with ILP-2 antibody at 1 µg/ml.
	Western blot analysis of Livin in Raji cell lysate with Livin antibody at 4µg/ml.
	Western blot analysis of NAIP in PC-3 lysate with NAIP antibody at (A) 0.5, (B) 1 and (C) 2 µg/ml.
	Western blot analysis of Survivin in MOLT4 cell lysate in (A) the presence or (B) absence of blocking peptide with Survivin antibody at 1 µg/ml.
	Western blot analysis of XIAP in human kidney tissue lysate with XIAP antibody at (A) 0.5, (B) 1 and (C) 2 µg/ml.

	Western blot analysis of cIAP in human lung tissue lysate with cIAP antibody at (A) 1, (B) 2 and (C) 4 µg/ml.
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	Immunohistochemistry of cIAP in human lung tissue with cIAP antibody at 10 µg/ml.
	Immunocytochemistry of ILP-2 in HepG2 cells with ILP-2 antibody at 10 µg/ml.
	Immunohistochemistry of Livin in human small intestine tissue with Livin antibody at 10 µg/ml.
	Immunocytochemistry of NAIP in A549 cells with NAIP antibody at 10 µg/ml.
	Immunocytochemistry of Survivin in MOLT4 cells with Survivin antibody at 10 µg/ml.

	Immunohistochemistry of XIAP in human kidney tissue with XIAP antibody at 2 µg/ml.
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APPLICATION:

These polyclonal antibodies can be used for detection of cIAP, ILP-2, Livin, NAIP, Survivin, and XIAP by immunoblot at 1 – 2 µg/ml. These antibodies can also be used at 2 – 10 µg/ml to detect their respective proteins via immunohistochemistry / immunocytochemistry. **For research use only.**

STORAGE:

Antibodies are supplied as affinity chromatography purified IgG, in PBS containing 0.02% sodium azide. Antibodies should be stored at -20°C.

POSITIVE CONTROLS:

cIAP antibody: Human Lung Tissue Lysate, **Catalog No. 1302.**

ILP-2 antibody: HepG2 Cell Lysate, **Catalog No. 1211.**

Livin antibody: Raji Cell Lysate, **Catalog No. 1207.**

NAIP antibody: PC-3 Cell Lysate, **Catalog No. 1216.**

Survivin antibody: MOLT4 Cell Lysate, **Catalog No. 1206.**

XIAP antibody: Human Kidney Tissue Lysate, **Catalog No. 1305.**

RELATED PRODUCTS:

cIAP peptide (CT), **Catalog No. 3325P.**

ILP-2 peptide (Irg), **Catalog No. 3017P.**

Livin peptide, **Catalog No. 2505P.**

NAIP peptide, **Catalog No. 3315P.**

Survivin peptide, **Catalog No. 2235P.**

XIAP peptide, **Catalog No. 3331P.**



REFERENCES:

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2. Verhagen AM, Coulson EJ, and Vaux DL. Inhibitor of apoptosis proteins and their relatives: IAPs and other BIRPs. *Genome Biol.* 2001; 2:reviews3009.1-reviews3009.10.
3. Roy N, Mahadevan MS, McLean M, et al. The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell* 1995; 80:167-78.
4. Deveraux QL, Takahashi R, Savesan GS, et al. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 1997; 388:300-4.
5. Rothe M, Pan M-G, Henzel WJ, et al. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* 1995; 83:1243-52.
6. Kasof GM, Gomes BC. Livin, a novel inhibitor of apoptosis protein family member. *J. Biol. Chem.* 2001; 276:3238-46.
7. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat. Med.* 1997; 3:917-21.
8. Richter BW, Mir SS, Eiben LJ, et al. Molecular cloning of ILP-2, a novel member of the inhibitor of apoptosis protein family. *Mol. Cell Biol.* 2001; 21:4292-301.
9. Hauser HP, Bardroff M, Pyrowolakis G, et al. A giant ubiquitin-conjugating enzyme related to IAP apoptosis inhibitors. *J. Cell Biol.* 1998; 141:1415-22.

WESTERN BLOT PROTOCOL:

- 1) Load 20 to 25 micrograms of whole cell lysate per lane in an SDS-PAGE mini gel.
- 2) Run at 20 mA per gel until the dye front is close to the bottom.
- 3) Transfer the proteins to a nitrocellulose membrane at 250 mA in transfer buffer for 1-4 h, depending on the size of the target protein.
- 4) Incubate the blot with blocking buffer (5% non-fat dry milk in TBS) overnight at 4°C or 2 hr at room temperature (RT).
- 5) Incubate the blot with primary antibody (diluted 1:250 to 1:1000 in blocking buffer) for 1 hr in blocking buffer at RT.
- 6) Wash the blot 3 x 10 min in washing buffer (TBS containing 0.1% Tween 20) with shaking.
- 7) Incubate blot with anti-rabbit IgG-HRP conjugate (diluted 1:10,000 -1:20,000 in blocking buffer) for 1 h in blocking buffer at RT.
- 8) Wash 3 x 10 min in washing buffer with shaking.
- 9) Drain washing buffer, add ECL solution and develop for 1 min.
- 10) Expose to X-ray film for 1 to 30 min.

MATERIALS NEEDED:

Nitrocellulose membrane
Non-fat dry milk
Tween-20
Antibody detection kit

TBS:

- 125 mM NaCl
- 25 mM Tris pH 8.0
- 0.1% Tween 20

SDS/Running Buffer:

- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS

Transfer Buffer:

- 20 mM Tris
- 150 mM Glycine
- 20% methanol
- 0.038% SDS