



Anti-IAP (Inhibitor of Apoptosis Protein) Sampler Set

CATALOG No: 54315

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). Up-regulation of these proteins inhibits the normal apoptotic process and thus may be involved in human diseases such as cancer.

SAMPLER CONTENTS:

Anti-cIAP, **Catalog No. 54173 (25 µg)**.

Anti-ILP-2, **Catalog No. 54117 (25 µg)**.

Anti-Livin, **Catalog No. 54108 (25 µg)**.

Anti-NAIP, **Catalog No. 54170 (25 µg)**.

Anti-Survivin (CT), **Catalog No. 53872 (25 µg)**.

Anti-XIAP, **Catalog No. 54174 (25 µg)**.

SOURCE AND REACTIVITY:

Rabbit polyclonal antibodies were raised against peptides corresponding to amino acid sequences from each of the corresponding proteins. These polyclonal antibodies can be used for detection of cIAP, ILP-2, Livin, NAIP, Survivin, and XIAP by immunoblot at 1 – 2 µg/ml and respective proteins via immunohistochemistry at 2 – 10 µg/ml.

APPLICATION:

The following concentration ranges are recommended starting points for this product.

Immunoblot: 1 – 2 µg/ml

IHC: 2 – 10 µg/ml

WB

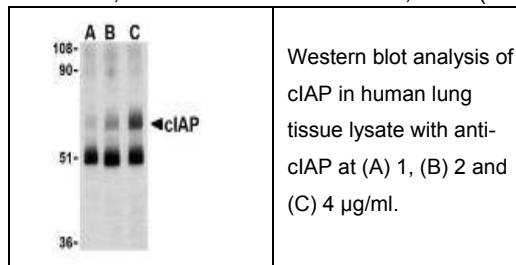
This product is for in vitro research purposes only.

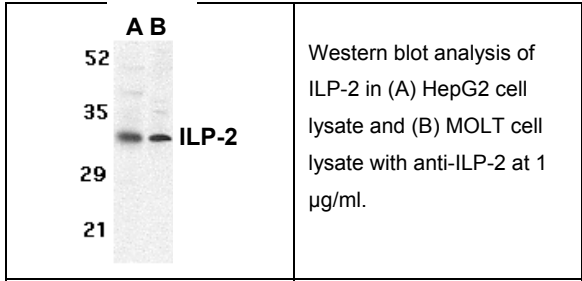
STORAGE:

The antibodies are supplied as affinity chromatography purified IgG, in 1X PBS containing 0.02% Sodium Azide. Store at 2-8 °C for up to 1 year. Avoid repeated freeze thaw cycles.

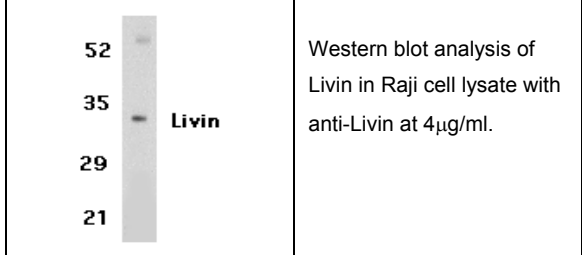
REFERENCES:

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2. Verhagen, AM. et al. *Genome Biol.* **2**, reviews3009.1(2001).
3. Roy, N. et al. *Cell* **80**, 167 (1995).
4. Deveraux, QL. et al. *Nature* **388**, 300 (1997).
5. Rothe, M. et al. *Cell* **83**, 1243 (1995).
6. Kasof, GM. et al. *J. Biol. Chem.* **276**, 3238 (2001).
7. Ambrosini, G. et al. *Nat. Med.* **3**, 917 (1997).
8. Richter, BW. et al. *Mol. Cell Biol.* **21**, 4292 (2001).
9. Hauser, HP. et al. *J. Cell Biol.* **141**, 1415 (1998).

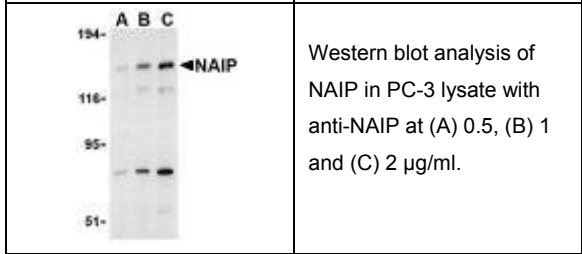




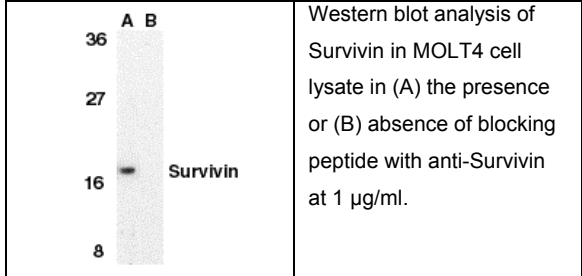
Western blot analysis of ILP-2 in (A) HepG2 cell lysate and (B) MOLT cell lysate with anti-ILP-2 at 1 µg/ml.



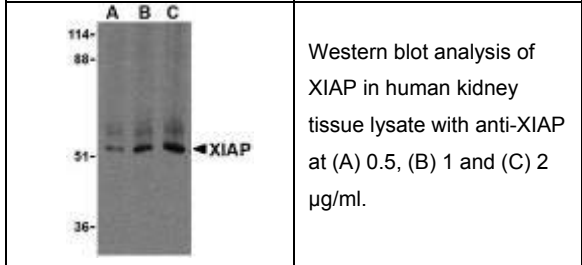
Western blot analysis of Livin in Raji cell lysate with anti-Livin at 4µg/ml.



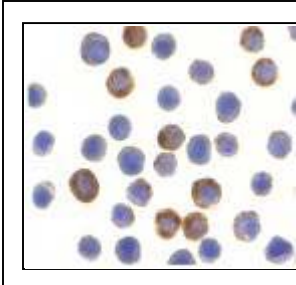
Western blot analysis of NAIP in PC-3 lysate with anti-NAIP at (A) 0.5, (B) 1 and (C) 2 µg/ml.



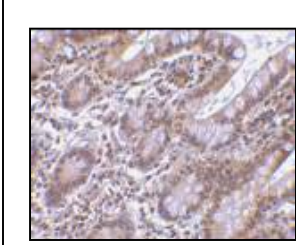
Western blot analysis of Survivin in MOLT4 cell lysate in (A) the presence or (B) absence of blocking peptide with anti-Survivin at 1 µg/ml.



Western blot analysis of XIAP in human kidney tissue lysate with anti-XIAP at (A) 0.5, (B) 1 and (C) 2 µg/ml.



Immunocytochemistry of ILP-2 in HepG2 cells with anti-ILP-2 at 10 µg/ml.



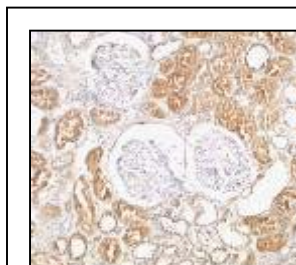
Immunohistochemistry of Livin in human small intestine tissue with anti-Livin at 10 µg/ml.



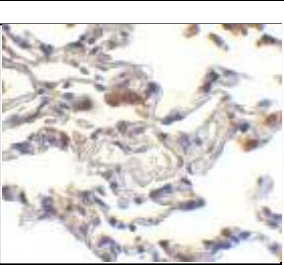
Immunocytochemistry of NAIP in A549 cells with anti-NAIP at 10 µg/ml.



Immunocytochemistry of Survivin in MOLT4 cells with anti-Survivin at 10 µg/ml.



Immunohistochemistry of XIAP in human kidney tissue with anti-XIAP at 2 µg/ml.



Immunohistochemistry of cIAP in human lung tissue with anti-cIAP at 10 µg/ml.

WESTERN BLOT PROTOCOL:

- 1.) Load 20-25 µg 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 hr, 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 hr 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 Sampler Set

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