c-IAP (Inhibitors of Apoptosis) 1 & 2 Rabbit Polyclonal Antibody

Cat# CY-P1041

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
-	WB	H, M, R	63-68 kDa	Rabbit IgG

Background

The cellular inhibitor of apoptosis 1 and 2 (c-IAP1 and c-IAP2) proteins were identified based on their homology to a family of viral and cellular IAP proteins known to bind to caspases and inhibit their activity (1). Both cIAP1 and cIAP2 contain three characteristic baculovirus IAP repeat domains that facilitate binding to caspases and other proteins (2, 3). In addition, they contain a caspase recruitment domain of unknown function and a RING finger domain that functions as an E3 ubiquitin ligase (4). The precise biologic roles of c-IAP1 and c-IAP2 are currently not known. Biochemical data have indicated that c-IAP1 and c-IAP2, initially thought to be caspase inhibitors (2), can bind to caspases but do not directly inhibit them (5). Instead, recent studies show c-IAP1 and c-IAP2 regulate TNF alpha -mediated NF-kappa B signaling and are required to protect cells against and TNF alpha - and Interleukin-1 beta -induced apoptosis (6-9).

Specificity/Sensitivity: This polyclonal antibody detects endogenous both c-IAP1 and c-IAP2 by western blotting.

Source/Purification: This polyclonal antibody is produced by immunizing rabbit with recombinant c-IAP1, corresponding full length of Rat c-IAP1, expressed in *E. coli*. IgG is purified by antigen-affinity chromatography.

Recommended Antibody Dilutions: Western blotting: 0.5 - 2 µg/mL.

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key:
WB: Western blotting, **IP:** Immunoprecipitation, **IHC:** Immunohistochemistry, **IC:** Immunocytochemistry, **F:** Flow cytometry, **E:** ELISA, **FP:** Fluorescence polarization assay

Species Cross-Reactivity Key:
H: Human, **M:** Mouse, **R:** Rat, **Hm:** Hamster, **Mk:** Monkey, **Mi:** Mink, **C:** Chicken, **X:** Xenopus, **Z:** Zebra fish (Species enclosed in parentheses are predicted to react based on 100 % sequence homology.)

References:

1. Liston P, et al. (1996) Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. Nature 379:349–353.

2. Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC (1997) The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBO J 16:6914–6925.

3. Samuel T, et al. (2006) Distinct BIR domains of cIAP1 mediate binding to and ubiquitination of tumor necrosis factor receptor-associated factor 2 and second mitochondrial activator of caspases. J Biol Chem 281:1080–1090.

4. Yang Y, Fang S, Jensen JP, Weissman AM, Ashwell JD (2000) Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. Science 288:874–877.

5. Eckelman BP, Salvesen GS (2006) The human anti-apoptotic proteins cIAP1 and cIAP2 bind but do not inhibit caspases. J Biol Chem 281:3254–3260.

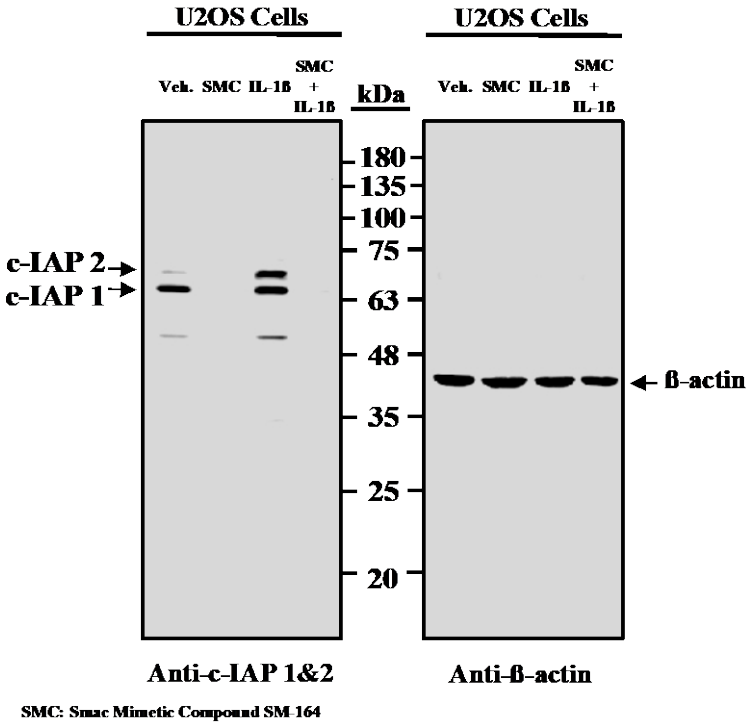
6. Vince JE, et al. (2007) IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 131:682–693.

7. Varfolomeev E, et al. (2007) IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. Cell 131:669–681.

8. Mahoney DJ, et al.(2008) Both cIAP1 and cIAP2 regulate TNF alpha-mediated NF-kappaB activation. Proc Natl Acad Sci U S A. 105:11778-83.

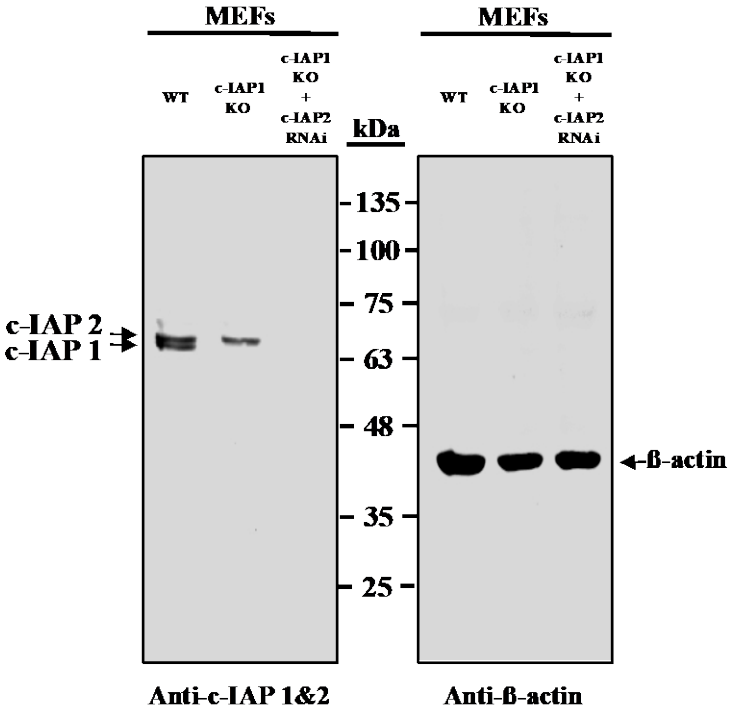
9. Cheung HH, et al.(2010) Smac mimetic compounds potentiate interleukin-1beta-mediated cell death. J Biol Chem. 285:40612-23.

Fig.1 WB analysis of endogenous human c-IAP 1&2 proteins*



SMC: Smac Mimetic Compound SM-164

Fig.2 WB analysis of endogenous mouse c-IAP 1&2 proteins*



* The data were kindly provided by Dr. Herman Cheung (Apoptosis Research Centre, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada).

Western blotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20 % methanol (pH 8.5)

SDS Sample Buffer (1X): 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

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 %).

10X TBS (Tris-buffered saline): 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).

Primary Antibody Dilution Buffer: 1X TBS, 0.1 % Tween-20 with 5 % blocking agent 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 %).

Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)

Wash Buffer TBS/T: 1X TBS, 0.1 % Tween-20

Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

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 per plate of 10 cm^2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3,000-5,000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute 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 ten-second exposure should indicate the proper exposure time.

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