Human cIAP-1/HIAP-2 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF8181

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human cIAP-1/HIAP-2. Does not cross-react with recombinant human cIAP-2 or XIAP.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human cIAP-1/HIAP-2 His2-Ser618 Accession # Q13490
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

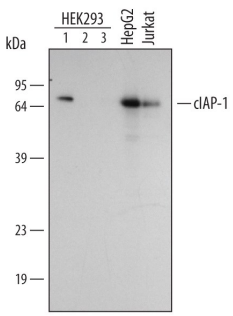
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	5 µg/mL	See Below
Knockout Validated	cIAP-1/HIAP-2 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in cIAP-1/HIAP-2 knockout HeLa cell line.	

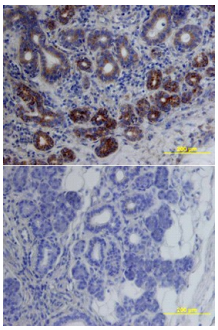
DATA

Western Blot



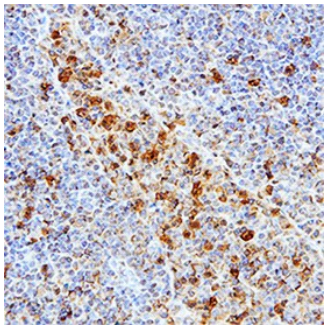
Detection of Human cIAP-1/HIAP-2 by Western Blot. Western blot shows lysates of HEK293 human embryonic kidney cell line transfected with human cIAP-1 (lane 1), human cIAP-2 (lane 2), or non-transfected (lane 3). PVDF membrane was probed with 0.5 µg/mL Goat Anti-Human cIAP-1/HIAP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8181) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). For additional reference, lystates of HepG2 human hepatocellular carcinoma cell line and Jurkat human acute T cell leukemia cell line were included. A specific band for cIAP-1/HIAP-2 was detected at approximately 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunohistochemistry



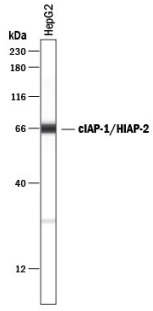
cIAP-1/HIAP-2 in Human Lymphoma. cIAP-1/HIAP-2 was detected in immersion fixed paraffin-embedded sections of human lymphoma using Goat Anti-Human cIAP-1/HIAP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8181) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Immunohistochemistry



cIAP-1/HIAP-2 in Human Lymph Node. cIAP-1/HIAP-2 was detected in immersion fixed paraffin-embedded sections of human lymph node using Goat Anti-Human cIAP-1/HIAP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8181) at 10 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human cIAP-1/HIAP-2 by Simple Western™. Simple Western lane view shows lysates of HepG2 human hepatocellular carcinoma cell line, loaded at 0.5 mg/mL. A specific band was detected for cIAP-1/HIAP-2 at approximately 66 kDa (as indicated) using 5 µg/mL of Goat Anti-Human cIAP-1/HIAP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8181) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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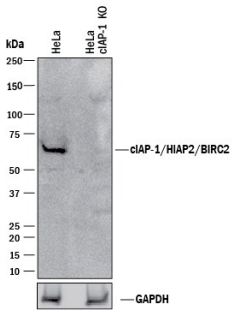
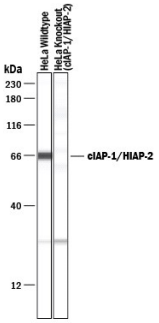
Knockout Validated



Western Blot Shows Human cIAP-1/HIAP-2 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and cIAP-1/HIAP-2 knockout HeLa cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human cIAP-1/HIAP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8181) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for cIAP-1/HIAP-2 at approximately 68 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Knockout Validated



Specificity of Human cIAP-1/HIAP-2 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma parental cell line and cIAP-1/HIAP-2 knockout HeLa cell line (KO), loaded at 0.2 mg/mL. A specific band was detected for cIAP-1/HIAP-2 at approximately 66 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. Goat Anti-Human cIAP-1/HIAP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8181) was used at 5 µg/mL followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

cIAP-1 (also known as BIR2, MIHB and HIAP-2) is a member of the inhibitor of apoptosis (IAP) family of proteins that inhibit the proteolytic activity of mature caspases. cIAP-1 has 3 BIR (baculovirus inhibitor of apoptosis) domains, a RING finger domain, and a caspase recruitment domain (CARD). cIAP-1 inhibits caspases by interaction of the BIR domain with the active caspase. Caspase activity may be restored through interactions with the Reaper like motif on mitochondrial proteins such as SMAC/Diablo or HTRA-2/Omi. cIAP-1 is reported to be cleaved by caspases in fetal rat hepatocytes treated with TGF-β.

References:

1. Roy, N. *et al.* (1997) EMBO J. **23**:6914.
2. Deveraux, Q. *et al.* (1997) Nature **388**:300.
3. Deveraux, Q. and J. Reed (1999) Genes & Develop. **13**:239.
4. Herrera, B. *et al.* (2002) FEBS Letters **520**:93.

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