

# Biotin Conjugated Anti-clAP1 Antibody [PSH06-71] - Detector

## HA723283B



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	ELISA(Det), ELISA
<b>Clone number:</b>	PSH06-71

**Description:** Multi-functional protein which regulates not only caspases and apoptosis, but also modulates inflammatory signaling and immunity, mitogenic kinase signaling, and cell proliferation, as well as cell invasion and metastasis. Acts as an E3 ubiquitin-protein ligase regulating NF-kappa-B signaling and regulates both canonical and non-canonical NF-kappa-B signaling by acting in opposite directions: acts as a positive regulator of the canonical pathway and suppresses constitutive activation of non-canonical NF-kappa-B signaling. The target proteins for its E3 ubiquitin-protein ligase activity include: RIPK1, RIPK2, RIPK3, RIPK4, CASP3, CASP7, CASP8, TRAF2, DIABLO/SMAC, MAP3K14/NIK, MAP3K5/ASK1, IKBKG/NEMO, IKBKE and MXD1/MAD1. Can also function as an E3 ubiquitin-protein ligase of the NEDD8 conjugation pathway, targeting effector caspases for neddylation and inactivation. Acts as an important regulator of innate immune signaling via regulation of Toll-like receptors (TLRs), Nodlike receptors (NLRs) and RIG-I like receptors (RLRs), collectively referred to as pattern recognition receptors (PRRs). Protects cells from spontaneous formation of the ripoptosome, a large multi-protein complex that has the capability to kill cancer cells in a caspase-dependent and caspase-independent manner. Suppresses ripoptosome formation by ubiquitinating RIPK1 and CASP8. Can stimulate the transcriptional activity of E2F1. Plays a role in the modulation of the cell cycle.

<b>Conjugate:</b>	Biotin-conjugated
<b>Immunogen:</b>	Recombinant protein within human clAP1 aa 201-618 (HA211301).
<b>Positive control:</b>	Recombinant Human clAP1 protein (HA211301).
<b>Subcellular location:</b>	Cytoplasm, Nucleus.
<b>Database links:</b>	SwissProt: Q13490 Human   Q62210 Mouse Entrez Gene: 60371 Rat

### Recommended Dilutions:

<b>ELISA(Det)</b>	Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH06-72] to Human clAP1 antibody (Capture) (HA723280) or Rabbit monoclonal [PSH02-10] to Human clAP1 antibody (Capture) (HA723281) and Recombinant Human clAP1 protein (HA211301) as the standard. The reference range value is 62.5-8,000 pg/ml.
<b>ELISA</b>	Use at an assay dependent concentration.

<b>Storage Buffer:</b>	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% ProClin300.
<b>Storage Instruction:</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.
<b>Purity:</b>	Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

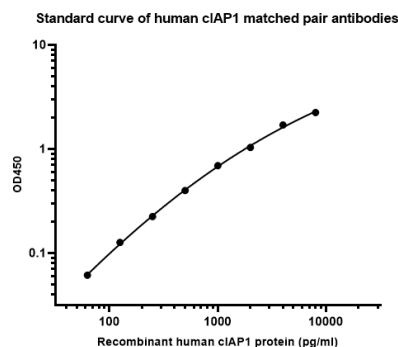
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## Images

**Fig1:** Sandwich ELISA analysis of human cIAP1 matched pair antibodies

Capture: HA723280, cIAP1 Rabbit mAb [PSH06-72]

Detector: HA723282, cIAP1 Rabbit mAb [PSH06-71]

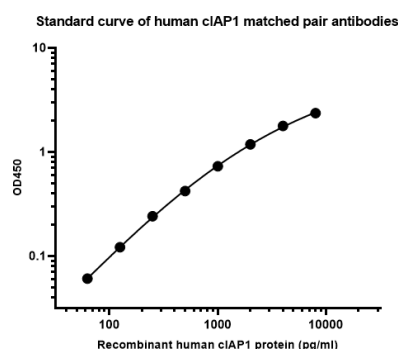


Elisa assay was performed by coating wells of a 96-well plate with 50  $\mu$ l per well of capture antibody (HA723280) diluted in carbonate/bicarbonate buffer, at a concentration of 2  $\mu$ g/ml overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150  $\mu$ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human cIAP1 protein (HA211301) starting from 8,000 pg/ml to 0 pg/ml and detect antibody (HA723283B, 0.2  $\mu$ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 50  $\mu$ l per well of SA-HRP for 0.5 hour at 30 $^{\circ}$ C with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

**Fig2:** Sandwich ELISA analysis of human cIAP1 matched pair antibodies

Capture: HA723281, cIAP1 Rabbit mAb [PSH02-10]

Detector: HA723282, cIAP1 Rabbit mAb [PSH06-71]



Elisa assay was performed by coating wells of a 96-well plate with 50  $\mu$ l per well of capture antibody (HA723281) diluted in carbonate/bicarbonate buffer, at a concentration of 2  $\mu$ g/ml overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150  $\mu$ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human cIAP1 protein (HA211301) starting from 8,000 pg/ml to 0 pg/ml and detect antibody (HA723283B, 0.2  $\mu$ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 50  $\mu$ l per well of SA-HRP for 0.5 hour at 30 $^{\circ}$ C with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

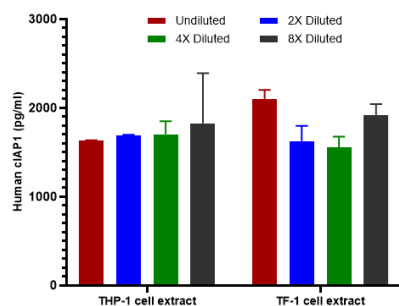
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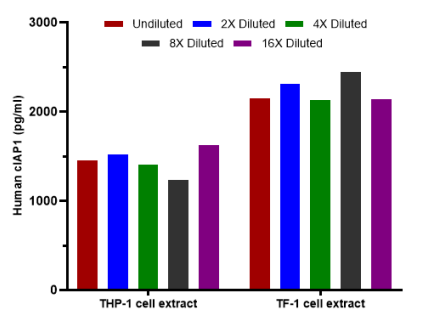
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**Fig3:** Interpolated concentrations of native cIAP1 in THP-1 and TF-1 extract samples based on a 1,000 µg/ml extract load.

Capture: HA723280, cIAP1 Rabbit mAb [PSH06-72]  
 Detector: HA723282, cIAP1 Rabbit mAb [PSH06-71]

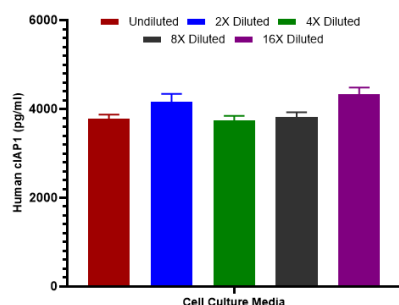
The concentrations of cIAP1 were measured in duplicates, interpolated from the cIAP1 standard curve and corrected for sample dilution. Undiluted samples are THP-1 extract 25% and TF-1 extract 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean cIAP1 concentration was determined to be 1,712 pg/ml in THP-1 extract and 1,800 pg/ml in TF-1 extract.



**Fig4:** Interpolated concentrations of native cIAP1 in THP-1 and TF-1 extract samples based on a 1,000 µg/mL extract load.

Capture: HA723281, cIAP1 Rabbit mAb [PSH02-10]  
 Detector: HA723282, cIAP1 Rabbit mAb [PSH06-71]

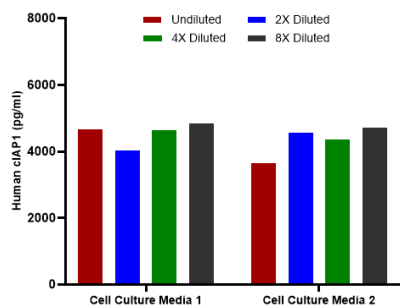
The concentrations of cIAP1 were interpolated from the cIAP1 standard curves and corrected for sample dilution. Undiluted samples are THP-1 extract 50% and TF-1 extract 50%. The mean cIAP1 concentration was determined to be 1,450 pg/ml in THP-1 extract and 2,235 pg/ml in TF-1 extract.



**Fig5:** Interpolated concentrations of spiked cIAP1 in human cell culture media samples.

Capture: HA723280, cIAP1 Rabbit mAb [PSH06-72]  
 Detector: HA723282, cIAP1 Rabbit mAb [PSH06-71]

The concentrations of cIAP1 were measured in triplicates, interpolated from the cIAP1 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=3).



**Fig6:** Interpolated concentrations of spiked cIAP1 in human cell culture media samples.

Capture: HA723281, cIAP1 Rabbit mAb [PSH02-10]

Detector: HA723282, cIAP1 Rabbit mAb [PSH06-71]

The concentrations of cIAP1 were interpolated from the cIAP1 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 50%.

**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

### Background References

1. Zadoroznyj A et al. Cytoplasmic and Nuclear Functions of cIAP1. *Biomolecules*. 2022 Feb
2. Yang X et al. TRIM56 promotes malignant progression of glioblastoma by stabilizing cIAP1 protein. *J Exp Clin Cancer Res*. 2022 Dec

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