Anti-Human cIAP2 Antibody [PSH09-39] - BSA and Azide free (Capture/Detector)

HA723099

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Det), ELISA(Cap)
Clone number:	PSH09-39

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, IKBKE, TRAF1, and BCL10. 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.

Immunogen: Recombinant protein within Human cIAP2 aa 246-604.

Positive control: Recombinant Human cIAP2 Protein (HA210619).

Subcellular location: Cytoplasm. Nucleus.

Database links: SwissProt: Q13489 Human

Recommended Dilutions:

ELISA(Det)
ELISA(Cap)

Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH09-38] to Human cIAP2 antibody (Capture) (HA723098) and Recombinant Human cIAP2 protein (HA210619) as the standard. The reference range value is 78-20,000pg/ml.

Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH09-40] to Human cIAP2 antibody (Detector) (HA723100) and Recombinant Human cIAP2 protein (HA210619) as the standard. The reference range value is 78-20,000pg/ml.

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

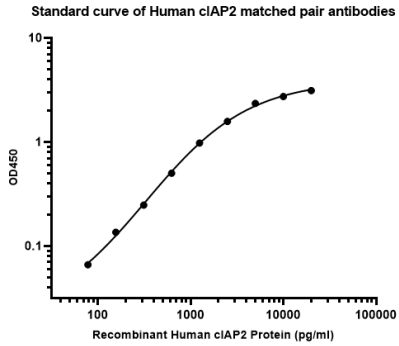

Fig1: Sandwich ELISA analysis of human cIAP2 matched pair antibodies.

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA723098) diluted in carbonate/bicarbonate buffer, at a concentration of 5ug/ml overnight at 4℃. Wells of the plate were washed, blocked with 150 µl 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human cIAP2 Protein (HA210619) starting from 20000 pg/ml to 0 pg/ml and detect antibody (HA723099, Biotin, 0.2 µg/ml) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 100 µl per well of SA-HRP for 0.5 hour at 30℃ 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.

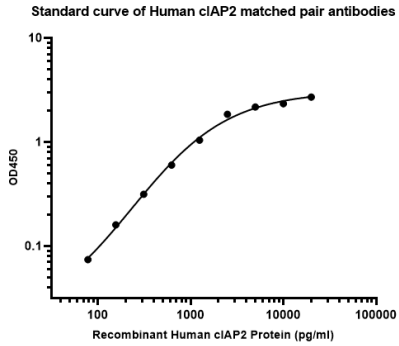

Fig2: Sandwich ELISA analysis of human cIAP2 matched pair antibodies.

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA723099) diluted in carbonate/bicarbonate buffer, at a concentration of 5ug/ml overnight at 4℃. Wells of the plate were washed, blocked with 150 µl 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human cIAP2 Protein (HA210619) starting from 20000 pg/ml to 0 pg/ml and detect antibody (HA723100, Biotin, 0.2 µg/ml) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 100 µl per well of SA-HRP for 0.5 hour at 30℃ 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.

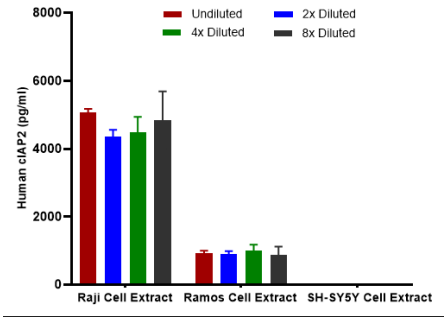

Fig3: Interpolated concentrations of native cIAP2 in Raji, Ramos and SH-SY5Y extract samples based on a 1000 µg/ml extract load.

Interpolated concentration of native cIAP2 was measured in duplicate at different sample concentrations and interpolated from the cIAP2 standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean cIAP2 concentration was determined to be 4,595 pg/mL in Raji and 927 pg/mL in Ramos cell extract, There was no detectable signal in SH-SY5Y cell extract.

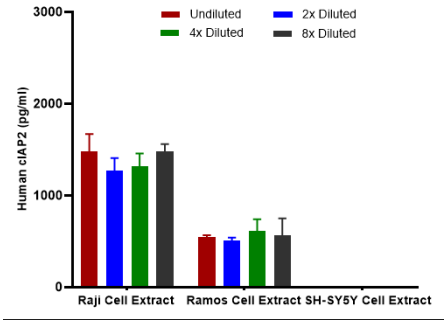

Fig4: Interpolated concentrations of native cIAP2 in Raji, Ramos and SH-SY5Y extract samples based on a 1000 µg/ml extract load.

Interpolated concentration of native cIAP2 was measured in duplicate at different sample concentrations and interpolated from the cIAP2 standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean cIAP2 concentration was determined to be 1,390 pg/mL in Raji and 561 pg/mL in Ramos cell extract, There was no detectable signal in SH-SY5Y cell extract.

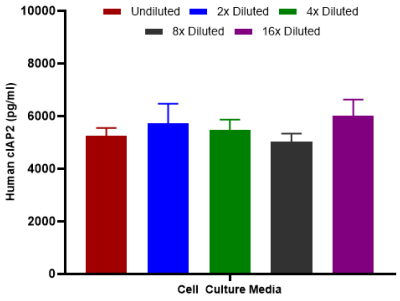

Fig5: Interpolated concentrations of spiked cIAP2 in cell extract samples.

The concentrations of cIAP2 were measured in duplicates, interpolated from the cIAP2 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell extract 0.2mg/ml. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

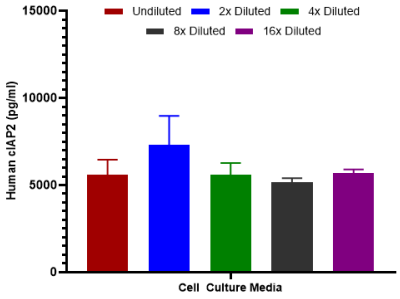

Fig6: Interpolated concentrations of spiked cIAP2 in cell culture media samples.

The concentrations of cIAP2 were measured in duplicates, interpolated from the cIAP2 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=2).

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

Background References

1. Bertrand M.J., Lippens S., Staes A., Gilbert B., Roelandt R., De Medts J., Gevaert K., Declercq W., Vandenabeele P. cIAP1/2 are direct E3 ligases conjugating diverse types of ubiquitin chains to receptor interacting proteins kinases 1 to 4 (RIP1-4). PLoS ONE 6:E22356-E22356 (2011)
2. Zhou A.Y., Shen R.R., Kim E., Lock Y.J., Xu M., Chen Z.J., Hahn W.C. IKKepsilon-mediated tumorigenesis requires K63-linked polyubiquitination by a cIAP1/cIAP2/TRAF2 E3 ubiquitin ligase complex. Cell Rep. 3:724-733 (2013)