

# Biotin Conjugated Anti-Human Caspase-9 Antibody [PSH17-20] - Detector

## HA723907B



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human
<b>Applications:</b>	ELISA(Det), ELISA
<b>Clone number:</b>	PSH17-20

**Description:** Caspase-9 is an enzyme that in humans is encoded by the CASP9 gene. It is an initiator caspase, critical to the apoptotic pathway found in many tissues. Caspase-9 homologs have been identified in all mammals for which they are known to exist, such as Mus musculus and Pan troglodytes. Caspase-9 belongs to a family of caspases, cysteine-aspartic proteases involved in apoptosis and cytokine signalling. Apoptotic signals cause the release of cytochrome c from mitochondria and activation of apaf-1 (apoptosome), which then cleaves the pro-enzyme of caspase-9 into the active dimer form. Regulation of this enzyme occurs through phosphorylation by an allosteric inhibitor, inhibiting dimerization and inducing a conformational change.

**Conjugate:** Biotin-conjugated

**Immunogen:** Recombinant protein within Human Caspase-9 aa 1-330 (HA211015).

**Positive control:** Recombinant Human Caspase-9 protein (HA211015).

**Subcellular location:** Apoptosome, caspase complex, cytoplasm, cytosol, mitochondrion, nucleus, protein-containing complex.

**Database links:** SwissProt: P55211 Human

### Recommended Dilutions:

**ELISA(Det)** Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH17-19] to Human Caspase-9 antibody (Capture) (HA723906) and Recombinant Human Caspase-9 protein (HA211015) as the standard. The reference range value is 19.5-5,000 pg/mL.

**ELISA** Use at an assay dependent concentration.

**Storage Buffer:** PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% ProClin300.

**Storage Instruction:** 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.

**Purity:** Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

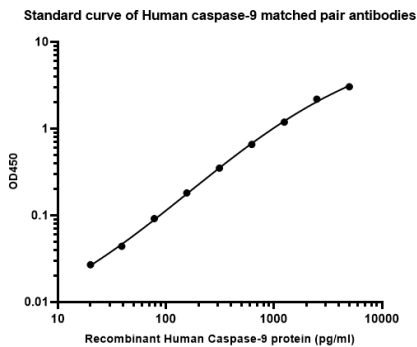
Service mail:support@huabio.cn

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

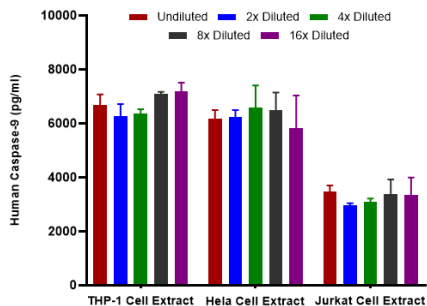
**Fig1:** Sandwich ELISA analysis of Human Caspase-9 matched pair antibodies

Capture: HA723906, Human Caspase-9 Rabbit mAb [PSH17-19]  
Detector: HA723907, Human Caspase-9 Rabbit mAb [PSH17-20]



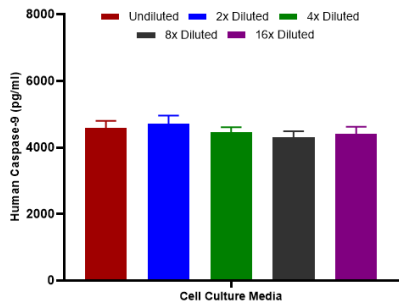
Elisa assay was performed by coating wells of a 96-well plate with 100  $\mu$ l per well of capture antibody (HA723906) diluted in carbonate/bicarbonate buffer, at a concentration of 2ug/ml overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150  $\mu$ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human Caspase-9 protein (HA211015) starting from 5,000 pg/ml to 0 pg/ml and detect antibody (HA723907, Biotin, 0.2  $\mu$ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 100  $\mu$ 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:** Interpolated concentrations of native caspase-9 in THP-1, Hela and Jurkat cell extract samples based on a 1000  $\mu$ g/ml extract load.



Capture: HA723906, Human Caspase-9 Rabbit mAb [PSH17-19]  
Detector: HA723907, Human Caspase-9 Rabbit mAb [PSH17-20]

Interpolated concentration of native caspase-9 was measured in duplicate at different sample concentrations and interpolated from the caspase-9 standard curves. The interpolated dilution factor corrected values were plotted (mean  $\pm$  SD, n=2). The mean caspase-9 concentration was determined to be 6,717 pg/mL in THP-1, 6,267 pg/mL in Hela and 3,257 pg/mL in Jurkat cell extract.



**Fig3:** Interpolated concentrations of spiked caspase-9 in cell culture media samples.

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Detector: HA723907, Human Caspase-9 Rabbit mAb [PSH17-20]

The concentrations of caspase-9 were measured in duplicates, interpolated from the caspase-9 standard curves and corrected for sample dilution. Diluted samples are as follows: 50% cell culture media with FBS. 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. Li J et al. Caspase-9 inhibition triggers Hsp90-based chemotherapy-mediated tumor intrinsic innate sensing and enhances antitumor immunity. J Immunother Cancer. 2023 Dec
2. Sever AIM et al. Activation of caspase-9 on the apoptosome as studied by methyl-TROSY NMR. Proc Natl Acad Sci U S A. 2023 Dec

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