

HA723837



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap), ELISA(Det)
Clone number:	PSH16-39

Description: Receptor for TNFSF6/FASLG. The adapter molecule FADD recruits caspase CASP8 to the activated receptor. The resulting death-inducing signaling complex (DISC) performs CASP8 proteolytic activation which initiates the subsequent cascade of caspases (aspartate-specific cysteine proteases) mediating apoptosis. FAS-mediated apoptosis may have a role in the induction of peripheral tolerance, in the antigen-stimulated suicide of mature T-cells, or both. The secreted isoforms 2 to 6 block apoptosis.

Immunogen: Recombinant protein within Human Fas / CD95 aa 26-173 (HA211064).

Positive control: Recombinant Human Fas / CD95 protein (HA211064).

Subcellular location: Cell membrane, Membrane raft; Secreted.

Database links: SwissProt: P25445 Human

Recommended Dilutions:

ELISA(Cap) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH16-37] to Human Fas / CD95 antibody (Detector) (HA723835) and Recombinant Human Fas / CD95 protein (HA211064) as the standard. The reference range value is 15.6-2,000 pg/mL.

ELISA(Det) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH16-38] to Human Fas / CD95 antibody (Capture) (HA723836) and Recombinant Human Fas / CD95 protein (HA211064) as the standard. The reference range value is 15.6-2,000 pg/mL.

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at 2-8℃. Avoid freeze.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

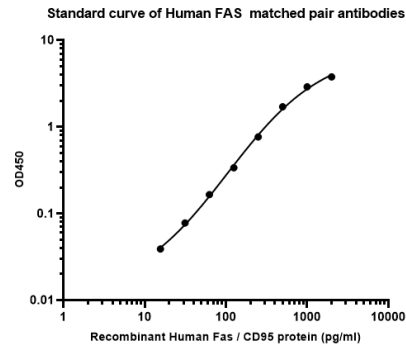
Service mail:support@huabio.cn

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Images

Fig1: Sandwich ELISA analysis of Human Fas / CD95 matched pair antibodies

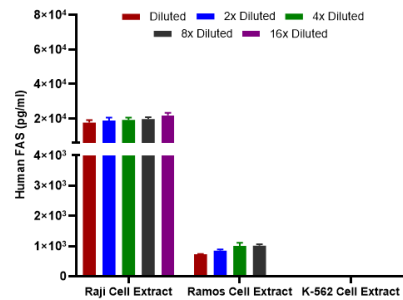
Capture: HA723836, Human Fas / CD95 Rabbit mAb [PSH16-38]
Detector: HA723837, Human Fas / CD95 Rabbit mAb [PSH16-39]



Elisa assay was performed by coating wells of a 96-well plate with 100 μ l per well of capture antibody (HA723836) diluted in carbonate/bicarbonate buffer, at a concentration of 2 μ g/mL overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150 μ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human Fas / CD95 protein (HA211064) starting from 2,000 pg/ml to 0 pg/ml and detect antibody (HA723837, Biotin, 0.2 μ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 100 μ 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 FAS in Raji,Ramos and K-562 Cell extract samples based on a 1000 μ g/ml extract load.

Capture: HA723836, Human Fas / CD95 Rabbit mAb [PSH16-38]
Detector: HA723837, Human Fas / CD95 Rabbit mAb [PSH16-39]



Interpolated concentration of native FAS was measured in duplicate at different sample concentrations. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean FAS concentration was determined to be 19,553 pg/ml in Raji Cell extract 898 pg/ml in Ramos Cell extract and undetectable in K-562 Cell extract.

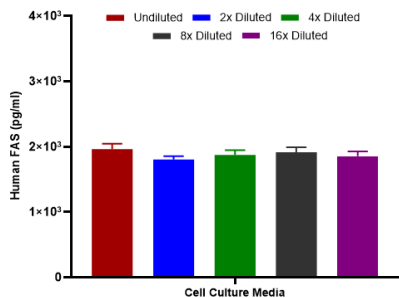


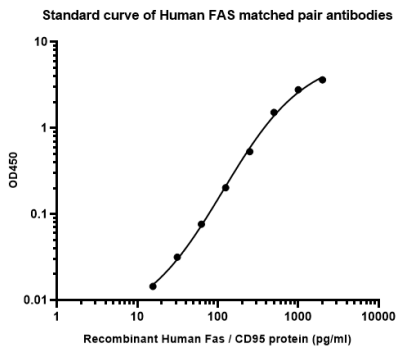
Fig3: Interpolated concentrations of spiked FAS in cell culture media samples.

Capture: HA723836, Human Fas / CD95 Rabbit mAb [PSH16-38]
Detector: HA723837, Human Fas / CD95 Rabbit mAb [PSH16-39]

The concentrations of FAS were measured in duplicates, interpolated from the FAS 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).

Fig4: Sandwich ELISA analysis of Human Fas / CD95 matched pair antibodies

Capture: HA723837, Human Fas / CD95 Rabbit mAb [PSH16-39]
Detector: HA723835, Human Fas / CD95 Rabbit mAb [PSH16-37]



Elisa assay was performed by coating wells of a 96-well plate with 100 μ l per well of capture antibody (HA723837) diluted in carbonate/bicarbonate buffer, at a concentration of 2 μ g/mL overnight at 4 $^{\circ}$ C. Wells of the plate were washed, blocked with 150 μ l 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human Fas / CD95 protein (HA211064) starting from 2,000 pg/ml to 0 pg/ml and detect antibody (HA723835, Biotin, 0.2 μ g/ml) for 1 hour at 30 $^{\circ}$ C with shaking. Then the plate was washed and incubated with 100 μ 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.

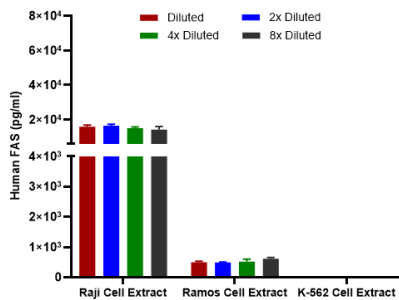


Fig5: Interpolated concentrations of native FAS in Raji,Ramos and K-562 Cell extract samples based on a 1000 µg/ml extract load.

Capture: HA723837, Human Fas / CD95 Rabbit mAb [PSH16-39]
Detector: HA723835, Human Fas / CD95 Rabbit mAb [PSH16-37]

Interpolated concentration of native FAS was measured in duplicate at different sample concentrations. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean FAS concentration was determined to be 15,453 pg/ml in Raji Cell extract 529 pg/ml in Ramos Cell extract and undetectable in K-562 Cell extract.

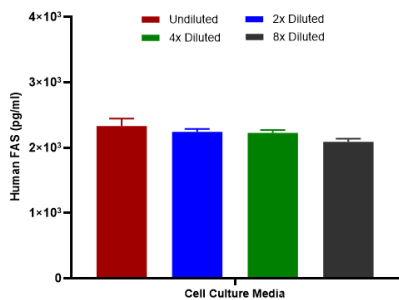


Fig6: Interpolated concentrations of spiked FAS in cell culture media samples.

Capture: HA723837, Human Fas / CD95 Rabbit mAb [PSH16-39]
Detector: HA723835, Human Fas / CD95 Rabbit mAb [PSH16-37]

The concentrations of FAS were measured in duplicates, interpolated from the FAS 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. Haymour L et al. CD95 (Fas) and CD95L (FasL)-mediated non-canonical signaling pathways. Biochim Biophys Acta Rev Cancer. 2023 Nov
2. Galli G et al. Fas/CD95 Signaling Pathway in Damage-Associated Molecular Pattern (DAMP)-Sensing Receptors. Cells. 2022 Apr

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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