Anti-Human Granzyme A Antibody [PSH09-35] - BSA and Azide free (Detector)

HA723093

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Det)
Clone number:	PSH09-35

Description: Abundant protease in the cytosolic granules of cytotoxic T-cells and NK-cells which activates caspase-independent pyroptosis when delivered into the target cell through the immunological synapse. Once delivered into the target cell, acts by catalyzing cleavage of gasdermin-B (GSDMB), releasing the pore-forming moiety of GSDMB, thereby triggering pyroptosis and target cell death. Cleaves the nucleosome assembly protein SET after 'Lys-189', which disrupts its nucleosome assembly activity and allows the SET complex to translocate into the nucleus to nick and degrade the DNA. Cytolytic T lymphocytes (CTL) and natural killer (NK) cells share the remarkable ability to recognize, bind, and lyse specific target cells. They are thought to protect their host by lysing cells bearing on their surface 'nonself' antigens, usually peptides or proteins resulting from infection by intracellular pathogens. The protein described here is a T cell- and natural killer cell-specific serine protease that may function as a common component necessary for lysis of target cells by cytotoxic T lymphocytes and natural killer cells.

Immunogen: Recombinant protein within Human Granzyme A aa 29-262.

Positive control: Recombinant Human Granzyme A protein (HA210916).

Subcellular location: Secreted.

Database links: SwissProt: P12544 Human

Recommended Dilutions:

ELISA(Det) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH09-34] to Human Granzyme A antibody (Capture) (HA723092) and Recombinant Human Granzyme A protein (HA210916) as the standard. The reference range value is 15.6-2,000 pg/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

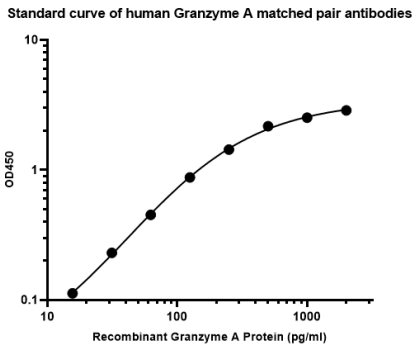

Fig1: Sandwich ELISA analysis of human Granzyme A matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 50 µl per well of capture antibody (HA723092) diluted in carbonate/bicarbonate buffer, at a concentration of 2 µg/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 Granzyme A protein (HA210916) starting from 1,000 pg/ml to 0 pg/ml and detect antibody (HA723093, Biotin, 0.2 µg/ml) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 50 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.

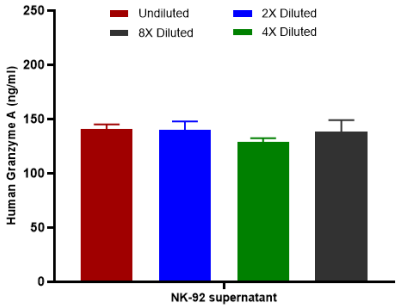

Fig2: Interpolated concentrations of native Granzyme A in NK-92 cell culture supernatant.

The concentrations of Granzyme A were measured in duplicates, interpolated from the Granzyme A standard curve and corrected for sample dilution. Undiluted samples are NK-92 cell culture supernatant 1%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Granzyme A concentration was determined to be 137.0 ng/ml in NK-92 cell culture supernatant.

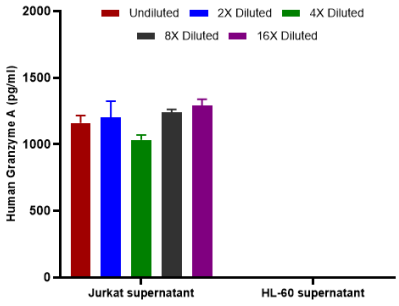

Fig3: Interpolated concentrations of native Granzyme A in Jurkat and HL-60 cell culture supernatant.

The concentrations of Granzyme A were measured in duplicates, interpolated from the Granzyme A standard curve and corrected for sample dilution. Undiluted samples are Jurkat cell culture supernatant 100% and HL-60 cell culture supernatant 100%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Granzyme A concentration was determined to be 1,184.5 pg/ml in Jurkat cell culture supernatant and undetectable in HL-60 cell culture supernatant.

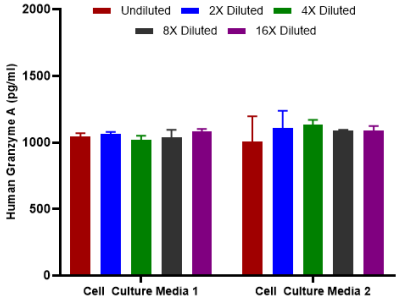

Fig4: Interpolated concentrations of spiked Granzyme A in human cell culture media samples.

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

Background References

1. Zhou Z., He H., Wang K., Shi X., Wang Y., Su Y., Wang Y., Li D., Liu W., Zhang Y., Shen L., Han W., Shen L., Ding J., Shao F. Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. Science 368:0-0 (2020)
2. Oltra S.S., Colomo S., Sin L., Perez-Lopez M., Lazaro S., Molina-Crespo A., Choi K.H. Distinct GSDMB protein isoforms and protease cleavage processes differentially control pyroptotic cell death and mitochondrial damage in cancer cells. Cell Death Differ. 30:1366-1381 (2023)