Anti-Human CD73 Antibody [PSH08-15] - BSA and Azide free (Capture)

HA722957

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap)
Clone number:	PSH08-15

Description: Catalyzes the hydrolysis of nucleotide monophosphates, releasing inorganic phosphate and the corresponding nucleoside, with AMP being the preferred substrate. The protein encoded by this gene is a plasma membrane protein that catalyzes the conversion of extracellular nucleotides to membrane-permeable nucleosides. The encoded protein is used as a determinant of lymphocyte differentiation. Defects in this gene can lead to the calcification of joints and arteries. Two transcript variants encoding different isoforms have been found for this gene. A condition characterized by adult-onset calcification of the lower extremity arteries, including the iliac, femoral and tibial arteries, and hand and foot capsule joints. Age of onset has been reported as early as the second decade of life, usually involving intense joint pain or calcification in the hands.

Immunogen: Recombinant protein within Human CD73 aa 27-549.

Positive control: Recombinant Human CD73 protein (HA210915).

Subcellular location: Cell membrane.

Database links: SwissProt: P21589 Human

Recommended Dilutions:

ELISA(Cap) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH08-16] to Human CD73 antibody (Detector) (HA722958) and recombinant Human CD73 protein as the standard (HA210915). The reference range value is 41.2-30.000 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

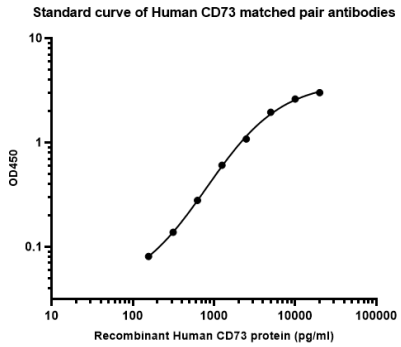

Fig1: Sandwich ELISA analysis of human CD73 matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 50 µl per well of capture antibody (HA722957) diluted in carbonate/bicarbonate buffer, at a concentration of 5 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 CD73 protein (HA210915) starting from 20,000 pg/ml to 0 pg/ml and detect antibody (HA722957, 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.

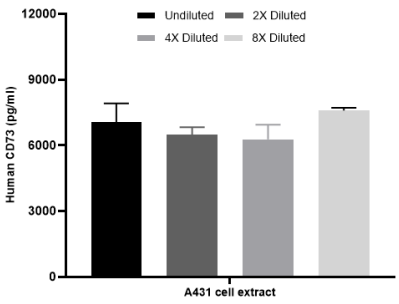

Fig2: Interpolated concentrations of native CD73 in A431 extract samples based on a 1,000 µg/ml extract load.

The concentrations of CD73 were measured in duplicates, interpolated from the CD73 standard curve and corrected for sample dilution. Undiluted samples are A431 extract 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CD73 concentration was determined to be 6,201 pg/ml in A431 extract.

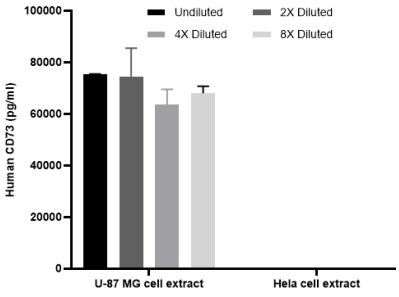

Fig3: Interpolated concentrations of native CD73 in U-87 MG and Hela extract samples based on a 1,000 µg/ml extract load.

The concentrations of CD73 were measured in duplicates, interpolated from the CD73 standard curve and corrected for sample dilution. Undiluted samples are U-87 MG extract 13% and Hela extract 100%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CD73 concentration was determined to be 70,356 pg/ml in U-87 MG extract and undetectable in Hela extract.

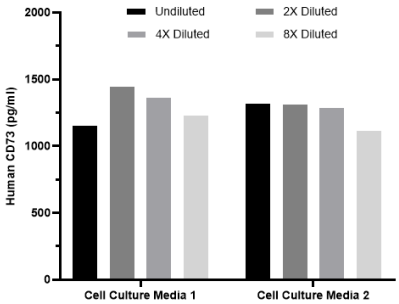

Fig4: Interpolated concentrations of spiked CD73 in human cell culture media samples.

The concentrations of CD73 were interpolated from the CD73 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 1 50%, cell culture media 2 50%.

Background References

1. Scaletti E., Huschmann F.U., Mueller U., Weiss M.S., Strater N. Substrate binding modes of purine and pyrimidine nucleotides to human ecto-5'-nucleotidase (CD73) and inhibition by their bisphosphonic acid derivatives. Purinergic Signal. 17:693-704 (2021)
2. Garavaglia S., Bruzzone S., Cassani C., Canella L., Allegrone G., Sturla L., Mannino E., Millo E., De Flora A., Rizzi M. The high-resolution crystal structure of periplasmic Haemophilus influenzae NAD nucleotidase reveals a novel enzymatic function of human CD73 related to NAD metabolism. Biochem. J. 441:131-141 (2012)