Anti-Human CD80 Antibody [PSH09-61] - BSA and Azide free (Capture)

HA723123

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap)
Clone number:	PSH09-61

Description: Costimulatory molecule that belongs to the immunoglobulin superfamily that plays an important role in T-lymphocyte activation. Acts as the primary auxiliary signal augmenting the MHC/TCR signal in naive T-cells together with the CD28 receptor which is constitutively expressed on the cell surface of T-cells. In turn, activates different signaling pathways such as NF-kappa-B or MAPK leading to the production of different cytokines. In addition, CD28/CD80 costimulatory signal stimulates glucose metabolism and ATP synthesis of T-cells by activating the PI3K/Akt signaling pathway. Acts also as a regulator of PDL1/PDCD1 interactions to limit excess engagement of PDL1 and its inhibitory role in immune responses. Expressed on B-cells, plays a critical role in regulating interactions between B-cells and T-cells in both early and late germinal center responses, which are crucial for the generation of effective humoral immune responses.

Immunogen: Recombinant protein within Human CD80 aa 35-242 (HA210994).

Positive control: Recombinant Human CD80 protein (HA210994).

Subcellular location: Cell membrane.

Database links: SwissProt: P33681 Human

Recommended Dilutions:

ELISA(Cap) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH09-62] to Human CD80 antibody (Detector) (HA723124) or Rabbit monoclonal [PSH09-63] to Human CD80 antibody (Detector) (HA723125) and Recombinant Human CD80 protein (HA210994) as the standard. The reference range value is 15.6-4,000 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

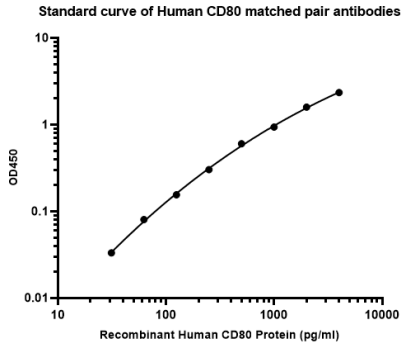

Fig1: Sandwich ELISA analysis of human CD80 matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA723123) diluted in carbonate/bicarbonate buffer, at a concentration of 2ug/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 CD80 protein (HA210994) starting from 4000 pg/ml to 0 pg/ml and detect antibody (HA723124, 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.

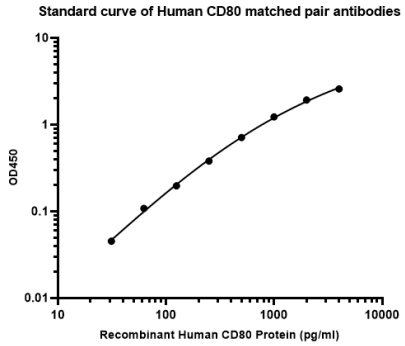

Fig2: Sandwich ELISA analysis of human CD80 matched pair antibodies

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA723123) diluted in carbonate/bicarbonate buffer, at a concentration of 2ug/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 CD80 protein (HA210994) starting from 4000 pg/ml to 0 pg/ml and detect antibody (HA723125, 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.

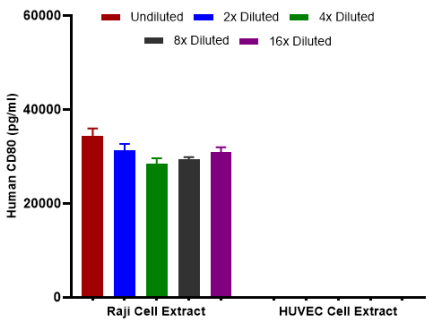

Fig3: Interpolated concentrations of native CD80 in Raji and HUVEC extract samples based on a 1000 µg/ml extract load.

Interpolated concentration of native CD80 was measured in duplicate at different sample concentrations and interpolated from the CD80 standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean CD80 concentration was determined to be 30,943 pg/mL in Raji cell extract, There was no detectable signal in HUVEC cell extract.

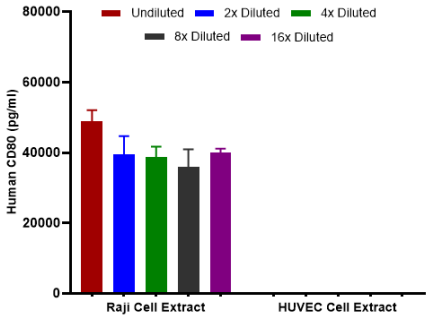

Fig4: Interpolated concentrations of native CD80 in Raji and HUVEC extract samples based on a 1000 µg/ml extract load.

Interpolated concentration of native CD80 was measured in duplicate at different sample concentrations and interpolated from the CD80 standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean CD80 concentration was determined to be 40,639 pg/mL in Raji cell extract, There was no detectable signal in HUVEC cell extract.

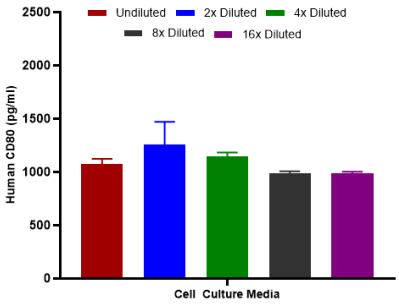

Fig5: Interpolated concentrations of spiked CD80 in cell culture media samples.

The concentrations of CD80 were measured in duplicates, interpolated from the CD80 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).

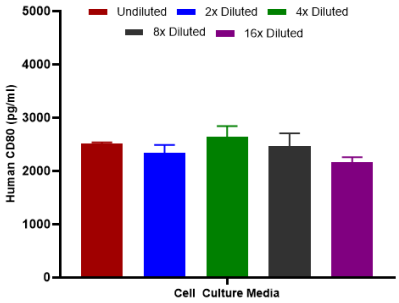

Fig6: Interpolated concentrations of spiked CD80 in cell culture media samples.

The concentrations of CD80 were measured in duplicates, interpolated from the CD80 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. Lu B., Sun Y.Y., Chen B.Y., Yang B., He Q.J., Li J., Cao J. zDHHC20-driven S-palmitoylation of CD80 is required for its costimulatory function. Acta Pharmacol. Sin. 0:0-0 (2024)
2. Kennedy A., Robinson M.A., Hinze C., Waters E., Williams C., Halliday N., Dovedi S., Sansom D.M. The CTLA-4 immune checkpoint protein regulates PD-L1:PD-1 interaction via transendocytosis of its ligand CD80. EMBO J. 42:e111556-e111556 (2023)