Anti-Human Siglec 9 Antibody [PSH11-89] - BSA and Azide free (Capture)

HA723397

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Cap)
Clone number:	PSH11-89

Description: Sialic acid-binding Ig-like lectin 9 is a protein that in humans is encoded by the SIGLEC9 gene. Putative adhesion molecule that mediates sialic-acid dependent binding to cells. Preferentially binds to alpha-2,3- or alpha-2,6-linked sialic acid. The sialic acid recognition site may be masked by cis interactions with sialic acids on the same cell surface. Expressed by peripheral blood leukocytes (neutrophils and monocytes but not eosinophils). Found in liver, fetal liver, bone marrow, placenta, spleen and in lower levels in skeletal muscle, fetal brain, stomach, lung, thymus, prostate, brain, mammary, adrenal gland, colon, trachea, cerebellum, testis, small intestine and spinal cordon.

Immunogen: Recombinant protein within Human Siglec 9 aa 18-348 (HA211074).

Positive control: Recombinant Human Siglec 9 protein (HA211074).

Subcellular location: Membrane.

Database links: SwissProt: Q9Y336 Human

Recommended Dilutions:

ELISA(Cap) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH11-90] to Human Siglec 9 antibody (Detector) (HA723398) and Rabbit monoclonal [PSH11-91] to Human Siglec 9 antibody (Detector) (HA723400) and Recombinant Human Siglec 9 protein (HA211074) as the standard. The reference range value is 31.3-4,000 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

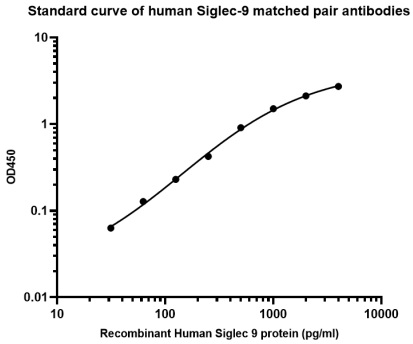

Fig1: Sandwich ELISA analysis of human Siglec 9 matched pair antibodies
Capture: HA723397, Siglec 9 Rabbit mAb [PSH11-89]
Detector: HA723398, Siglec 9 Rabbit mAb [PSH11-90]

Elisa assay was performed by coating wells of a 96-well plate with 50 µl per well of capture antibody (HA723397) 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 Siglec 9 protein (HA211074) starting from 4,000 pg/ml to 0 pg/ml and detect antibody (HA723398, 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.

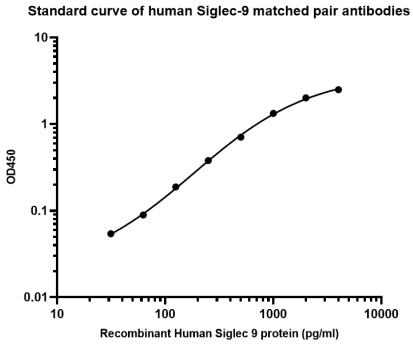

Fig2: Sandwich ELISA analysis of human Siglec 9 matched pair antibodies
Capture: HA723397, Siglec 9 Rabbit mAb [PSH11-89]
Detector: HA723400, Siglec 9 Rabbit mAb [PSH11-91]

Elisa assay was performed by coating wells of a 96-well plate with 50 µl per well of capture antibody (HA723397) 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 Siglec 9 protein (HA211074) starting from 4,000 pg/ml to 0 pg/ml and detect antibody (HA723400, 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.

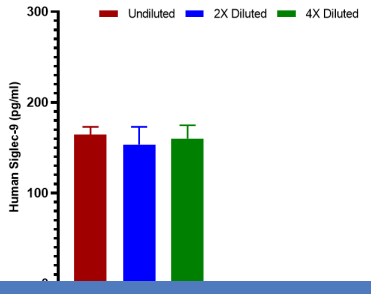

Fig3: Interpolated concentrations of native Siglec-9 in U937 and Jurkat extract samples based on a 1,000 µg/ml extract load.
Capture: HA723397, Siglec 9 Rabbit mAb [PSH11-89]
Detector: HA723398, Siglec 9 Rabbit mAb [PSH11-90]

The concentrations of Siglec-9 were measured in duplicates, interpolated from the Siglec-9 standard curve and corrected for sample dilution. Undiluted samples are U937 extract 100% and Jurkat extract 100%. The interpolated dilution factor corrected

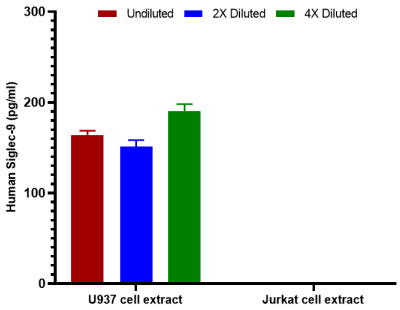

Fig4: Interpolated concentrations of native Siglec-9 in U937 and Jurkat extract samples based on a 1,000 µg/ml extract load.
Capture: HA723397, Siglec 9 Rabbit mAb [PSH11-89]
Detector: HA723400, Siglec 9 Rabbit mAb [PSH11-91]

The concentrations of Siglec-9 were measured in duplicates, interpolated from the Siglec-9 standard curve and corrected for sample dilution. Undiluted samples are U937 extract 100% and Jurkat extract 100%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Siglec-9 concentration was determined to be 168.5 pg/ml in U937 extract and undetectable in Jurkat extract.

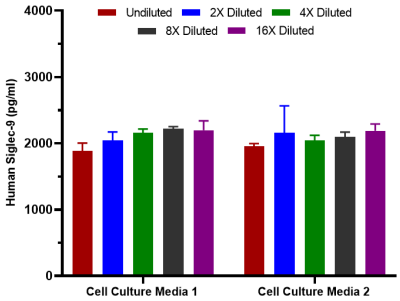

Fig5: Interpolated concentrations of spiked Siglec-9 in human cell culture media samples.
Capture: HA723397, Siglec 9 Rabbit mAb [PSH11-89]
Detector: HA723398, Siglec 9 Rabbit mAb [PSH11-90]

The concentrations of Siglec-9 were measured in duplicates, interpolated from the Siglec-9 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).

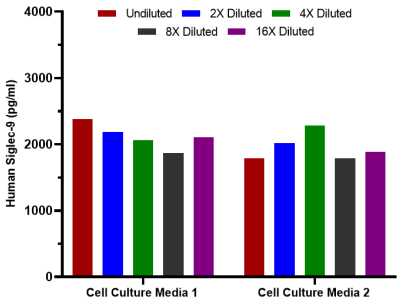

Fig6: Interpolated concentrations of spiked Siglec-9 in human cell culture media samples.
Capture: HA723397, Siglec 9 Rabbit mAb [PSH11-89]
Detector: HA723400, Siglec 9 Rabbit mAb [PSH11-91]

The concentrations of Siglec-9 were interpolated from the Siglec-9 standard curves and corrected for sample dilution. Undiluted samples are as follows: cell culture media 50%.

Background References

1. Mei Y et al. Siglec-9 acts as an immune-checkpoint molecule on macrophages in glioblastoma, restricting T-cell priming and immunotherapy response. Nat Cancer. 2023 Sep
2. Wang JHS et al. Development of Effective Siglec-9 Antibodies Against Cancer. Curr Oncol Rep. 2023 Jan