Anti-VISTA Antibody [PSH0-65] - BSA and Azide free

HA750642

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IF-Cell, FC, IF-Tissue
Molecular Wt:	Predicted band size: 34 kDa
Clone number:	PSH0-65

Description: V-domain Ig suppressor of T cell activation (VISTA) is a type I transmembrane protein that functions as an immune checkpoint and is encoded by the C10orf54 gene. VISTA is approximately 50kDa and belongs to the immunoglobulin superfamily and has one IgV domain. VISTA is part of the B7 family, is primarily expressed in white blood cells and its transcription is partially controlled by p53. There is evidence that VISTA can act as both a ligand and a receptor on T cells to inhibit T cell effector function and maintain peripheral tolerance. VISTA is produced at high levels in tumor-infiltrating lymphocytes, such as myeloid-derived suppressor cells and regulatory T cells, and its blockade with an antibody results in delayed tumor growth in mouse models of melanoma and squamous cell carcinoma. Monocytes from HIV-infected patients produce higher levels of VISTA compared to uninfected individuals. The increased VISTA levels correlated with an increase in immune activation and a decrease in CD4-positive T cells. There is an ongoing cancer immunotherapy clinical trial for a monoclonal antibody targeting VISTA in advanced cancer. Preliminary results of the phase I clinical trial show good safety tolerance and anti-cancer activity in patients with advanced tumours. Another ongoing clinical trial involves a small molecule that antagonizes the programmed death-ligands 1 and 2 (PD-L1 and PD-L2), and VISTA pathways in patients with advanced solid tumors or lymphomas.

Immunogen:	Recombinant protein within human VISTA aa 1-194 / 311.
Positive control:	Human lung tissue lysates, human brain tissue, human placenta tissue, human tonsil tissue, PBMC.
Subcellular location:	Cell membrane.
Database links:	SwissProt: Q9H7M9 Human
Recommended Dilutions:	
WB	1:1,000
IHC-P	1:200-1:1,000
IF-Cell	1:100
FC	1:500-1:1,000
IF-Tissue	1:500
Storage Buffer:	1*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

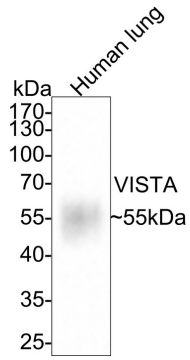

Fig1: Western blot analysis of VISTA on human lung tissue lysates with Rabbit anti-VISTA antibody (HA750642) at 1/1,000 dilution.

Lysates/proteins at 40 µg/Lane.

Predicted band size: 34 kDa
Observed band size: 55 kDa

Exposure time: 30 seconds;

10% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750642) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

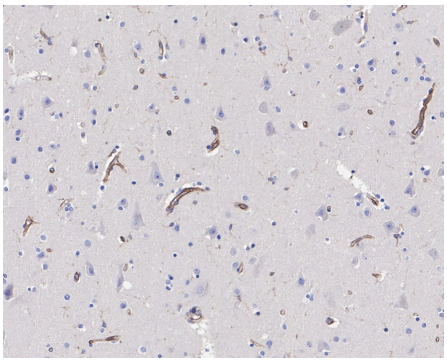

Fig2: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-VISTA antibody (HA750642) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750642) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

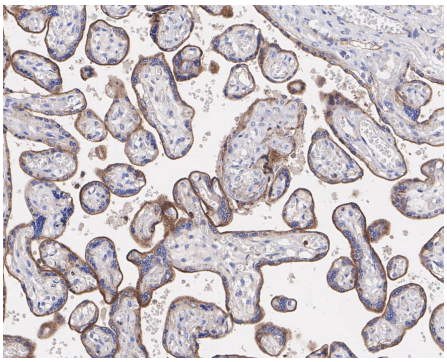

Fig3: Immunohistochemical analysis of paraffin-embedded human placenta tissue with Rabbit anti-VISTA antibody (HA750642) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750642) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

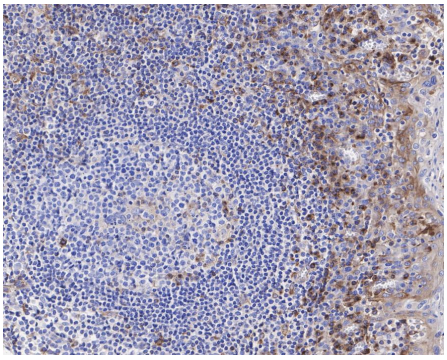

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-VISTA antibody (HA750642) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750642) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

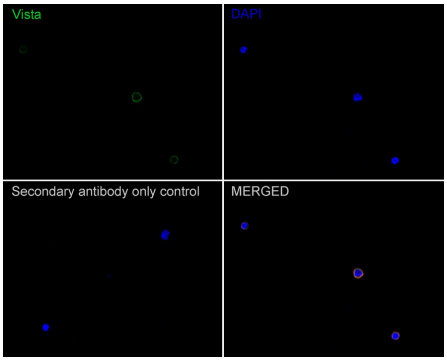

Fig5: Immunocytochemistry analysis of PBMC labeling VISTA with Rabbit anti-VISTA antibody (HA750642) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-VISTA antibody (HA750642) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

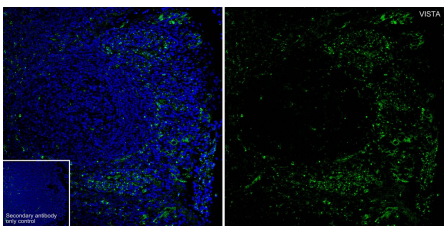

Fig6: Application: IF-Tissue

Species: Human

Site: tonsil

Sample: Paraffin-embedded section

Antibody concentration: 1/500

Background References

1. Yuan L et al. VISTA: A Mediator of Quiescence and a Promising Target in Cancer Immunotherapy. Trends Immunol. 2021 Mar
2. Huang X et al. VISTA: an immune regulatory protein checking tumor and immune cells in cancer immunotherapy. J Hematol Oncol. 2020 Jun