

# Anti-VISTA Antibody [PSH0-64]

HA721452



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human
<b>Applications:</b>	WB, IHC-P, IF-Tissue
<b>Molecular Wt:</b>	Predicted band size: 34 kDa
<b>Clone number:</b>	PSH0-64

**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:** A431 cell lysate, A375 cell lysate, K-562 cell lysate, TF-1 cell lysate, human lung tissue lysate, human brain tissue, human placenta tissue, human tonsil tissue.

**Subcellular location:** Cell membrane.

**Database links:** SwissProt: Q9H7M9 Human

**Recommended Dilutions:**

<b>WB</b>	1:1,000
<b>IHC-P</b>	1:1,000
<b>IF-Tissue</b>	1:500

**Storage Buffer:** PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

**Purity:** Protein A affinity purified.

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Orders:0086-571-88062880

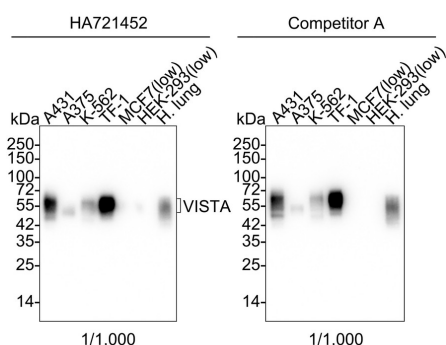
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## Images

**Fig1:** Western blot analysis of VISTA on different lysates with Rabbit anti-VISTA antibody (HA721452) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.



Lane 1: A431 cell lysate (20 µg/Lane)  
 Lane 2: A375 cell lysate (20 µg/Lane)  
 Lane 3: K-562 cell lysate (20 µg/Lane)  
 Lane 4: TF-1 cell lysate (20 µg/Lane)  
 Lane 5: MCF7 cell lysate (low expression) (20 µg/Lane)  
 Lane 6: HEK-293 cell lysate (low expression) (20 µg/Lane)  
 Lane 7: Human lung tissue lysate (30 µg/Lane)

Predicted band size: 34 kDa  
 Observed band size: 45-60 kDa (Glycosylated)

Exposure time: 30 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA721452) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

**Fig2:** Western blot analysis of VISTA on different lysates with Rabbit anti-VISTA antibody (HA721452) at 1/1,000 dilution.

Lane 1: A431-si NT cell lysate  
 Lane 2: A431-si VISTA cell lysate

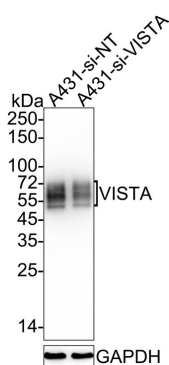
Lysates/proteins at 10 µg/Lane.

Predicted band size: 34 kDa  
 Observed band size: 45-60 kDa (Glycosylated)

Exposure time: 28 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA721452) at 1/1,000 dilution was used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



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**Fig3:** Western blot analysis of VISTA on different lysates with Rabbit anti-VISTA antibody (HA721452) at 1/1,000 dilution.

Lane 1: THP-1 cell lysate

Lane 2: THP-1 cell lysate treated with deglycosylation

Lysates/proteins at 20 µg/Lane.

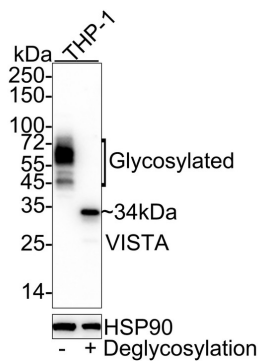
Predicted band size: 34 kDa

Observed band size: 34/45-60 kDa (Glycosylated)

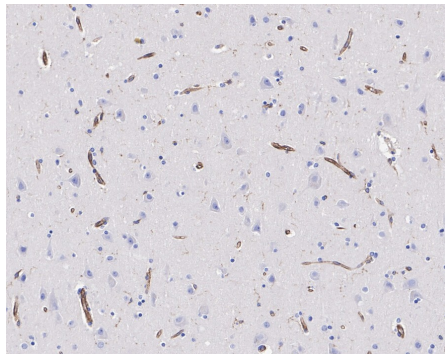
Exposure time: 40 seconds; ECL: K1801;

4-20% 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 (HA721452) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

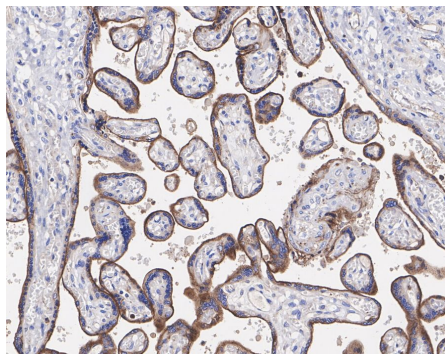


**Fig4:** Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-VISTA antibody (HA721452) at 1/1,000 dilution.



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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721452) 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:** Immunohistochemical analysis of paraffin-embedded human placenta tissue with Rabbit anti-VISTA antibody (HA721452) 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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721452) 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.

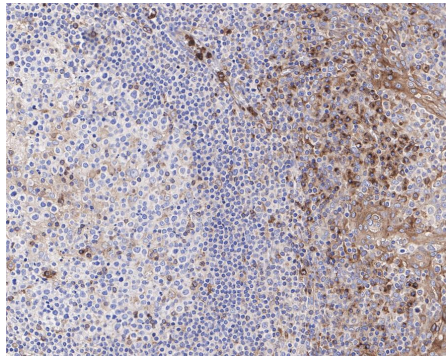
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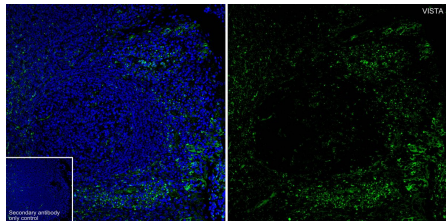
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**Fig6:** Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-VISTA antibody (HA721452) at 1/1,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA721452) 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.



**Fig7:** Application: IF-Tissue

Species: Human

Site: tonsil

Sample: Paraffin-embedded section

Antibody concentration: 1/500

**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

### 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

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