inhibitory Immune Checkpoint Antibody Sampler Kit

HAK21171

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
CTLA-4 [HA721269]	20µl	IHC-P,mIHC	H,M	25 kDa
LAG-3 [HA721358]	20µl	IHC-P,IF-Cell,WB,mIHC	H	57 kDa
PD-L1 [HA721176]	20µl	IHC-P,mIHC,WB	H	33 kDa
PD1 [ET1606-41]	20µl	WB,IHC-P	H	32 kDa
TIM 3 [HA723854]	20µl	WB,IHC-P,IP	H,M,R	33 kDa
VISTA [HA721452]	20µl	WB,IHC-P,IF-Tissue	H	34 kDa
HRP-Goat anti-Rabbit IgG UltraPolymer [HA1119]	5ml	IHC-P	Rab	

Description: The Inhibitory Immune Checkpoint Antibody Sampler Kit offers a cost-effective solution for detecting key proteins involved in inhibitory immune checkpoint pathways.The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Background The Inhibitory Immune Checkpoint pathway is a critical immune regulatory mechanism that tumors exploit to evade immune destruction.
CTLA-4 functions as a critical regulator of early T-cell activation, primarily within lymph nodes. PD-1/PD-L1 represents a major pathway driving T-cell exhaustion, and its blockade has become a cornerstone of modern immunotherapy. LAG-3, a transmembrane member of the immunoglobulin superfamily, inhibits T-cell maturation, differentiation, and proliferation. This suppression ultimately facilitates immune evasion in tumor cells. TIM-3 serves as a key marker of T-cell exhaustion, and ligand binding to TIM-3 inhibits effector cytokine production. VISTA is predominantly expressed on myeloid cells and acts to suppress T-cell activation within the tumor microenvironment.

Database links: UniProt ID: P16410, P09793, P18627, Q9NZQ7, Q15116, Q8TDQ0, Q8VIM0, P0C0K5, Q9H7M9

Images

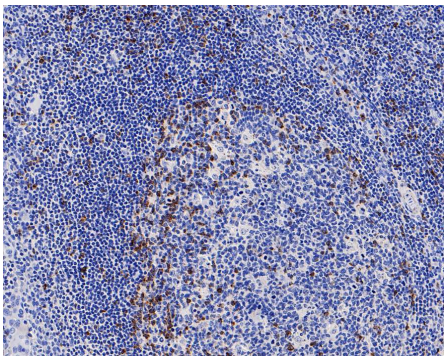

Fig1: Immunohistochemical analysis of paraffin-embedded human tonsils tissue with Rabbit anti-CTLA-4 antibody (HA721269) at 1/1,000 dilution.

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 (HA721269) 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.

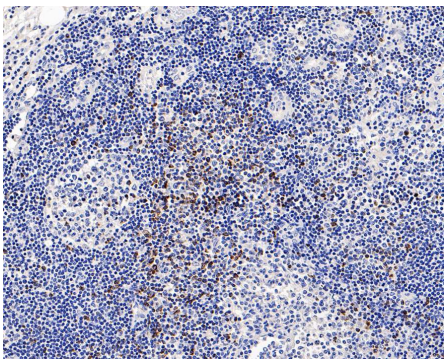

Fig2: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-CTLA-4 antibody (HA721269) at 1/1,000 dilution.

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 (HA721269) 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.

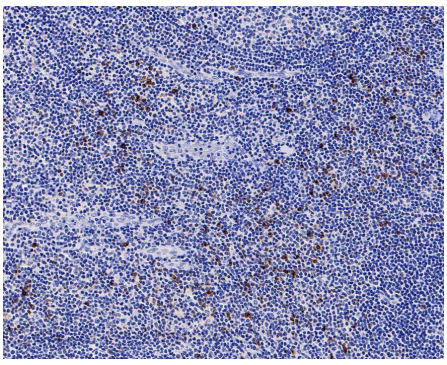

Fig3: Immunohistochemical analysis of paraffin-embedded human tonsils tissue with Rabbit anti-LAG-3 antibody (HA721358) at 1/200 dilution.

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 (HA721358) 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.

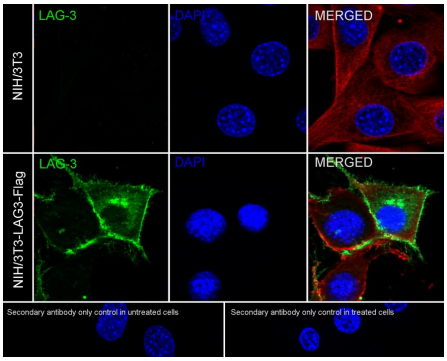

Fig4: Immunocytochemistry analysis of NIH/3T3 cells transfected with or without LAG-3 labeling LAG-3 with Rabbit anti-LAG-3 antibody (HA721358) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 1% BSA for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-LAG-3 antibody (HA721358) 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 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

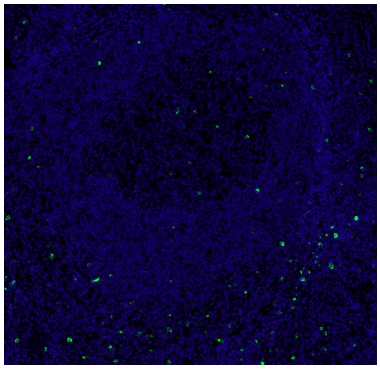

Fig5: mIHC analysis of human tonsils tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-LAG-3 antibody (HA721358) at 1/400 dilution. The immunostaining was performed with the IRISKitCmTSA Kit (900808). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

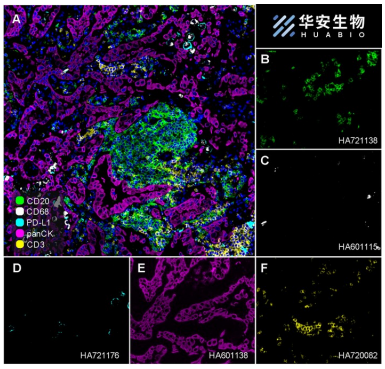

Fig6: Fluorescence multiplex immunohistochemical analysis of the human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-CD68 (HA601115, gray), anti-PD-L1 (HA721176, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human non-small cell lung cancer. Panel B: anti-CD20 stained on B cells. Panel C: anti-CD68 stained on macrophage M1 and macrophage M2. Panel D: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti-CD3 stained on T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of HA721138 (1/1,500 dilution), HA601115 (1/2,000 dilution), HA721176 (1/1,000 dilution), HA601138 (1/3,000 dilution), and HA720082 (1/500 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

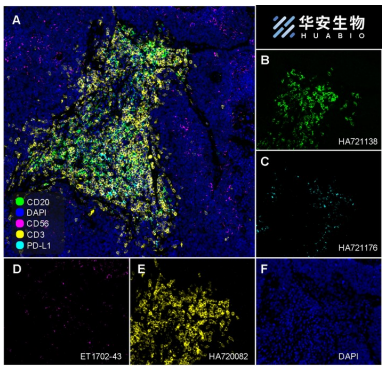

Fig7: Fluorescence multiplex immunohistochemical analysis of Tertiary Lymphoid Structures in Human Small Cell Lung Cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-PD-L1 (HA721176, cyan), anti-CD56 (ET1702-43, magenta) and anti-CD3 (HA720082, yellow) on tertiary lymphoid structures. Panel B: anti- CD20 stained on B cells. Panel C: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel D: anti-CD56 stained on NKT cells. Panel E: anti-CD3 stained on T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in four rounds of staining: in the order of HA721138 (1/1,500 dilution), HA721176 (1/1,000 dilution), ET1702-43 (1/1,000 dilution), and HA720082 (1/500 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

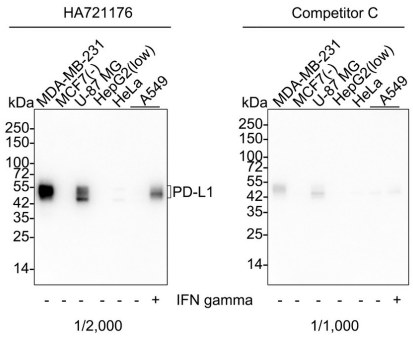

Fig8: Western blot analysis of PD-L1 on different lysates with Rabbit anti-PD-L1 antibody (HA721176) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: MDA-MB-231 cell lysate
Lane 2: MCF7 cell lysate (negative)
Lane 3: U-87 MG cell lysate
Lane 4: HepG2 cell lysate (low expression)
Lane 5: HeLa cell lysate
Lane 6: A549 cell lysate
Lane 7: A549 treated with 100ng/mL IFN gamma for 48 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 33 kDa
Observed band size: 45-50 kDa

Exposure time: 25 seconds; ECL: K1802;
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 (HA721176) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

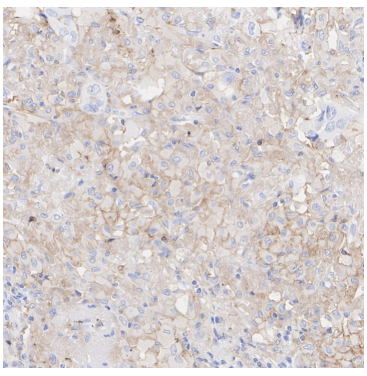

Fig9: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Rabbit anti-TIM 3 antibody (HA723854) at 1/1,000 dilution.

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 (HA723854) 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.

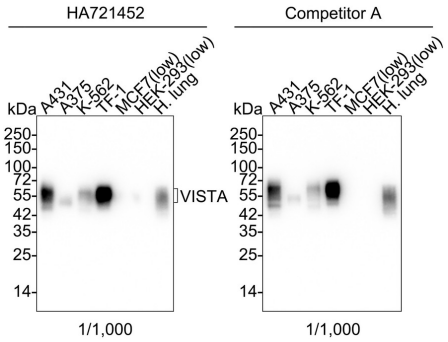

Fig10: Western blot analysis of VISTA on different lysates with Rabbit anti-VISTA antibody (HA721452) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.

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

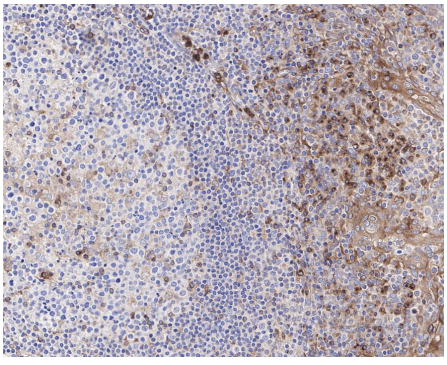

Fig11: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-VISTA antibody (HA721452) at 1/1,000 dilution.

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_2O 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.

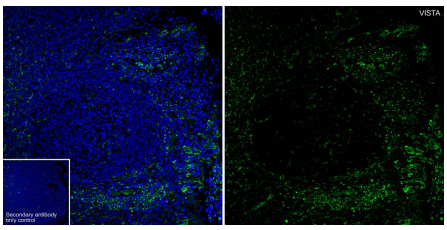

Fig12: Application: IF-Tissue

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. Burke KP, Patterson DG, Liang D, Sharpe AH. Immune checkpoint receptors in autoimmunity. Curr Opin Immunol. 2023 Feb;80:102283.
2. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012 Mar 22;12(4):252-64.
3. Curdy N, Lanvin O, Laurent C, Fournié JJ, Franchini DM. Regulatory Mechanisms of Inhibitory Immune Checkpoint Receptors Expression. Trends Cell Biol. 2019 Oct;29(10):777-790.
4. Borgeaud M, Sandoval J, Obeid M, Banna G, Michielin O, Addeo A, Friedlaender A. Novel targets for immune-checkpoint inhibition in cancer. Cancer Treat Rev. 2023 Nov;120:102614.
5. Dermani FK, Samadi P, Rahmani G, Kohlan AK, Najafi R. PD-1/PD-L1 immune checkpoint: Potential target for cancer therapy. J Cell Physiol. 2019 Feb;234(2):1313-1325.