Anti-LAG-3 Antibody [PS01-33]

HA721358

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	IHC-P, IF-Cell, WB
Molecular Wt:	Predicted band size: 57 kDa
Clone number:	PS01-33

Description: Lymphocyte-activation gene 3, also known as LAG-3, is a protein which in humans is encoded by the LAG3 gene. LAG3, which was discovered in 1990 and was designated CD223 (cluster of differentiation 223) after the Seventh Human Leucocyte Differentiation Antigen Workshop in 2000, is a cell surface molecule with diverse biologic effects on T cell function. It is an immune checkpoint receptor and as such is the target of various drug development programs by pharmaceutical companies seeking to develop new treatments for cancer and autoimmune disorders. In soluble form it is also being developed as a cancer drug in its own right. LAG3's main ligand is MHC class II, to which it binds with higher affinity than CD4. The protein negatively regulates cellular proliferation, activation, and homeostasis of T cells, in a similar fashion to CTLA-4 and PD-1 and has been reported to play a role in Treg suppressive function. Fibrinogen-like protein1 FGL1, a liver-secreted protein, is another (major) LAG3 functional ligand independent of MHC-II. LAG3 also helps maintain CD8+ T cells in a tolerogenic state and, working with PD-1, helps maintain CD8 exhaustion during chronic viral infection. LAG3 is known to be involved in the maturation and activation of dendritic cells.

Immunogen: Synthetic peptide.

Positive control: Human tonsil tissue, NIH/3T3 transfected with LAG3 cell lysate, NIH/3T3 cells transfected with LAG-3.

Subcellular location: Cell membrane; Secreted.

Database links: SwissProt: P18627 Human

Recommended Dilutions:

IHC-P	1:200
IF-Cell	1:100
WB	1:1,000

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

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

Purity: Protein A affinity purified.

Images

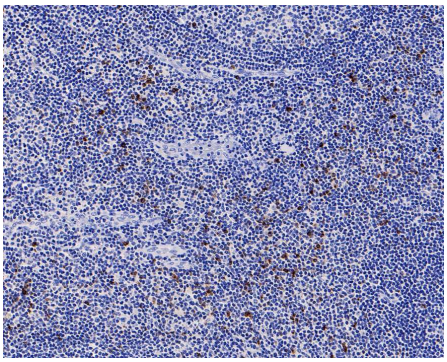

Fig1: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-LAG-3 antibody (HA721358) at 1/200 dilution.

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.

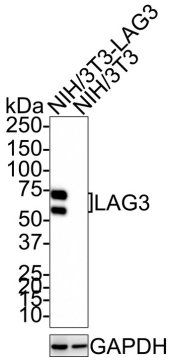

Fig2: Western blot analysis of LAG-3 on different lysates with Rabbit anti-LAG-3 antibody (HA721358) at 1/1,000 dilution.

Lane 1: NIH/3T3 transfected with LAG3 expression vector, whole cell lysate
Lane 2: NIH/3T3 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 57.4 kDa
Observed band size: 57/70 kDa

Exposure time: 2 minutes;

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 (HA721358) 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.

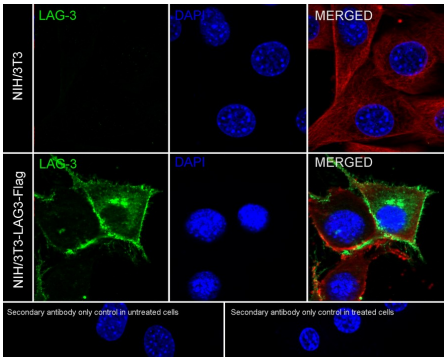

Fig3: Immunocytochemistry analysis of NIH/3T3 cells transfected with or without LAG-3 labeling LAG-3 with Rabbit anti-LAG-3 antibody (HA721358) at 1/100 dilution.

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.

Background References

1. Shi AP et al. Immune Checkpoint LAG3 and Its Ligand FGL1 in Cancer. Front Immunol. 2022 Jan
2. Guy C et al. LAG3 associates with TCR-CD3 complexes and suppresses signaling by driving co-receptor-Lck dissociation. Nat Immunol. 2022 May