Recombinant Human LAG-3 His-tag

Catalog Number: 10056-L3

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human LAG-3 protein Leu23-Leu450, with a C-terminal 6-His tag Accession # P18627
N-terminal Sequence Analysis	Leu23
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

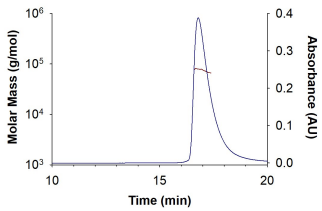
SDS-PAGE	57-63 kDa, reducing conditions
Activity	Measured by its ability to induce TNF-α secretion by JAWSII mouse immature dendritic cells. The ED_{50} for this effect is 0.2-1.2 µg/mL in the presence of a cross-linking antibody, Mouse Anti-polyHistidine Monoclonal Antibody (Catalog # MAB050R).
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 µg/mL in PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, ≤ -20 °C under sterile conditions after reconstitution.

DATA

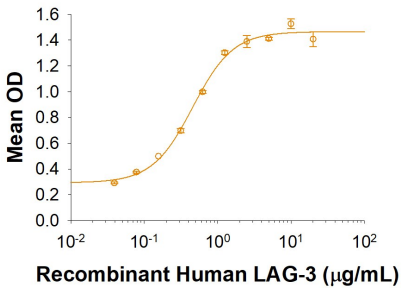
SEC-MALS



SEC-MALS Data	Result
Retention Time	16.6 - 17.4 min
MW - Predicted (Monomer)	47.0 kDa
MW - MALS	75.2 kDa
Polydispersity	1.004
System Suitability: BSA Monomer 66.4 ± 3.32 kDa	Pass

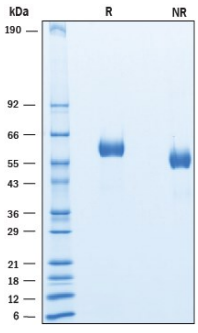
Recombinant Human LAG-3 His-tag Protein SEC-MALS. Recombinant human LAG-3 (Catalog # 10056-L3) has a molecular weight (MW) of 75.2 kDa as analyzed by SEC-MALS, suggesting that this protein is a monomer. MW may differ from predicted MW due to post-translational modifications (PTMs) present (i.e. Glycosylation).

Bioactivity



Recombinant Human LAG-3 His-tag Protein Bioactivity Recombinant Human LAG-3 induces TNF-a secretion in JAWSII mouse immature dendritic cells. The ED_{50} for this effect is 0.2-1.2 µg/mL in the presence of a cross-linking antibody, Mouse Anti-His Tag Monoclonal Antibody (Catalog # Catalog # MAB050R).

SDS-PAGE



Recombinant Human LAG-3 His-tag Protein SDS-PAGE 2 µg/lane of Recombinant Human LAG-3 was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 57-63 kDa and 55-60 kDa, respectively.

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BACKGROUND

LAG-3 (Lymphocyte activation gene-3), designated CD223, is a 70 kDa type I transmembrane protein that is a member of the immunoglobulin superfamily (IgSF) (1, 2). LAG-3 shares approximately 20% amino acid sequence homology with CD4, but has similar structure and binds to MHC class II with higher affinity, providing negative regulation of T cell receptor signaling (1, 2). Human LAG-3 cDNA encodes 525 amino acids (aa) that include a 28 aa signal sequence, a 422 aa extracellular domain (ECD) with four Ig-like domains, a transmembrane region and a highly charged cytoplasmic region. Within the ECD, human LAG-3 shares 70%, 67%, 76%, and 73% aa sequence identity with mouse, rat, porcine, and bovine LAG-3, respectively. LAG-3 is expressed on activated $CD4^+$ and CD8+ T cells, NK cells, and plasmacytoid dendritic cells (pDC), but not on resting T cells (1-3). LAG-3 on activated $CD4^+CD25^+$ Treg cells plays a role in their suppressive activity (4). LAG-3 limits the expansion of activated T cells and pDC in response to selected stimuli (3-5). A soluble 54 kDa form, sLAG-3, can be shed by metalloproteinases ADAM10 and TACE/ADAM17 (6, 7). While monomeric sLAG-3 itself may be inactive, shedding allows for normal T cell activation by removing negative regulation (7). Binding of a homodimerized sLAG-3/Ig fusion protein to MHC class II molecules induces maturation of immature DC, and secretion of cytokines such as IFN-gamma and TNF-alpha by type 1 cytotoxic $CD8^+$ T cells and NK cells (8, 9). sLAG-3/Ig has been used as a potential adjuvant to stimulate a cytotoxic anti-cancer immune response (9, 10). In mice, deletion of LAG-3 and another negative regulator, PD-1, facilitates anti-cancer response but also blocks self-tolerance and increases susceptibility to autoimmune diseases (11, 12). In humans, antibody-mediated down-regulation of LAG-3 and PD-1 allows more effective control of chronic malaria, while in NOD (non-obese diabetic) mice, deletion of LAG-3 alone accelerates diabetes (12-14). LAG-3 is an immune checkpoint protein that modulates T-cell activation and homeostasis and is a promising target for cancer immunotherapy (15, 16).

References:

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