Anti-CD86 Antibody [SJ20-00]

ET1606-50



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat
Applications: WB, IF-Cell, IP

Molecular Wt: Predicted band size: 38 kDa

Clone number: SJ20-00

Description: Cluster of Differentiation 86 (also known as CD86 and B7-2) is a protein constitutively

expressed on dendritic cells, Langerhans cells, macrophages, B-cells (including memory B-cells), and on other antigen-presenting cells. Along with CD80, CD86 provides costimulatory signals necessary for T cell activation and survival. Depending on the ligand bound, CD86 can signal for self-regulation and cell-cell association, or for attenuation of regulation and cell-cell disassociation. The CD86 gene encodes a type I membrane protein that is a member of the immunoglobulin superfamily. Alternative splicing results in two transcript variants encoding different isoforms. Additional transcript variants have been described, but their full-length sequences have not been determined. Roles of both CD80 and CD86 are studied in context of many pathologies. Selective inhibition of costimulatory inhibitors was examined in a model of allergic pulmonary inflammation and airway hyper-responsiveness (AHR). Since initial host response to Staphylococcus aureus, especially the immune response based on T cells, is a contributing factor in the pathogenesis of acute pneumonia, role of the CD80/CD86 pathway in pathogenesis was investigated. The costimulatory molecules were also investigated in context of Bronchial Astma, Treg in cancer, and

immunotherapy.

Immunogen: Synthetic peptide within Human CD86 aa 1-50 / 329.

Positive control: Daudi cell lysate, Raji cell lysate, Ramos cell lysate, K-562 cell lysate, Mouse lung tissue

lysate, Rat lung tissue lysate, Raji.

Subcellular location: Cell membrane.

Database links: SwissProt: P42081 Human | P42082 Mouse

Entrez Gene: 56822 Rat

Recommended Dilutions:

WB 1:5,000-1:20,000

IF-Cell 1:500

IP Use at an assay dependent concentration.

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℃ or -80℃. Avoid repeated freeze / thaw

cycles.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders: 0086-571-88062880 Technical: 0086-571-89986345

Service mail:support@huabio.cn



Images

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Fig1: Western blot analysis of CD86 on different lysates with Rabbit anti-CD86 antibody (ET1606-50) at 1/5,000 dilution.

Lane 1: Daudi cell lysate Lane 2: Raji cell lysate Lane 3: Ramos cell lysate Lane 4: K-562 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 38 kDa Observed band size: 70 kDa

Exposure time: 2 minutes 37 seconds;

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 (ET1606-50) at 1/5,000 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: Western blot analysis of CD86 on different lysates with Rabbit anti-CD86 antibody (ET1606-50) at 1/5,000 dilution.

Lane 1: Raji cell lysate (10 µg/Lane)

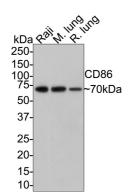
Lane 2: Mouse lung tissue lysate (20 µg/Lane) Lane 3: Rat lung tissue lysate (20 µg/Lane)

Predicted band size: 38 kDa Observed band size: 70 kDa

Exposure time: 1 minute;

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 (ET1606-50) at 1/5,000 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.



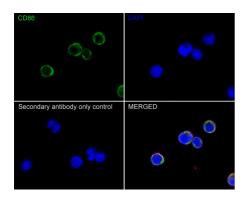


Fig3: Immunocytochemistry analysis of Raji cells labeling CD86 with Rabbit anti-CD86 antibody (ET1606-50) at 1/500 dilution.

Cells were fixed in 100% precooled methanol for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD86 antibody (ET1606-50) at 1/500 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor **M\$ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor † 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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

Background References

- 1. Li Y & Ding J Optimized generation of survivin-specific cytotoxic T lymphocytes against lung cancer. Mol Med Rep 12:2169-74 (2015).
- 2. Tarhini AA et al. Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. PLoS One 9:e87705 (2014).