

### Synonym

CD86,B7-2,B70,CD28LG2,LAB72,MGC34413

#### Source

Biotinylated Human B7-2, Fc, Avitag, premium grade(CD6-H82F5) is expressed from human 293 cells (HEK293). It contains AA Leu 26 - Pro 247 (Accession # P42081).

Predicted N-terminus: Leu 26

It is produced under our rigorous quality control system that incorporates a comprehensive set of tests including sterility and endotoxin tests. Product performance is carefully validated and tested for compatibility for cell culture use or any other applications in the early preclinical stage. When ready to transition into later clinical phases, we also offer a custom GMP protein service that tailors to your needs. We will work with you to customize and develop a GMP-grade product in accordance with your requests that also meets the requirements for raw and ancillary materials use in cell manufacturing of cell-based therapies.

## **Molecular Characterization**

B7-2(Leu 26 - Pro 247) Fc(Pro 100 - Lys 330)
P42081 P01857

This protein carries a human IgG1 Fc tag at the C-terminus, followed by an Avi tag (Avitag<sup>TM</sup>).

The protein has a calculated MW of 53.8 kDa. The protein migrates as 66-90 kDa under reducing (R) condition, and 130-150 kDa under non-reducing (NR) condition (SDS-PAGE) due to glycosylation.

## Labeling

Biotinylation of this product is performed using Avitag<sup>TM</sup> technology. Briefly, the single lysine residue in the Avitag is enzymatically labeled with biotin.

# **Protein Ratio**

Passed as determined by the HABA assay / binding ELISA.

### **Endotoxin**

Less than 0.01 EU per µg by the LAL method / rFC method.

#### **Sterility**

Negative

### Mycoplasma

Negative

#### **Purity**

>95% as determined by SDS-PAGE.

>95% as determined by SEC-MALS.

#### **Formulation**

Lyophilized from 0.22 µm filtered solution in 50 mM Tris, 100 mM Glycine, 25 mM Arginine, 150 mM NaCl, pH7.5 with trehalose as protectant.

Contact us for customized product form or formulation.

#### Reconstitution

Please see Certificate of Analysis for specific instructions.

For best performance, we strongly recommend you to follow the reconstitution protocol provided in the CoA.

#### Storage

For long term storage, the product should be stored at lyophilized state at -20°C or lower.

Please avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- -20°C to -70°C for 12 months in lyophilized state;
- -70°C for 3 months under sterile conditions after reconstitution.

**SDS-PAGE** 

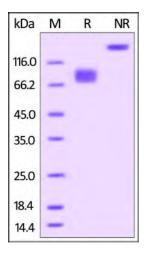
**SEC-MALS** 



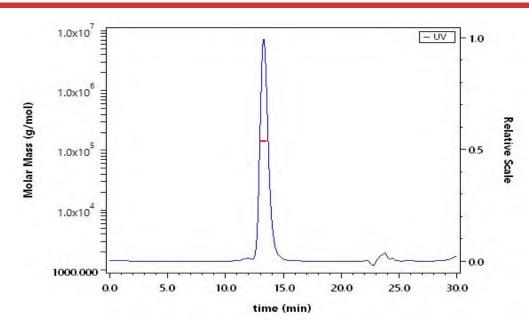
## Biotinylated Human B7-2 / CD86 Protein, Fc,Avitag™, premium grade

Catalog # CD6-H82F5





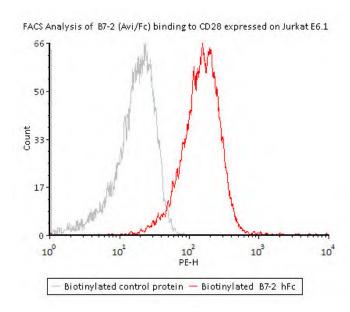
Biotinylated Human B7-2, Fc, Avitag, premium grade on SDS-PAGE under reducing (R) and non-reducing (NR) conditions. The gel was stained with Coomassie Blue. The purity of the protein is greater than 95%.



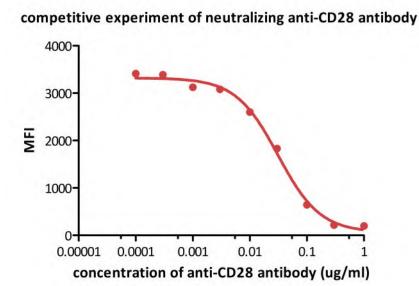
The purity of Biotinylated Human B7-2, Fc, Avitag, premium grade (Cat. No. CD6-H82F5) is more than 95% and the molecular weight of this protein is around 135-165 kDa verified by SEC-MALS.

Report

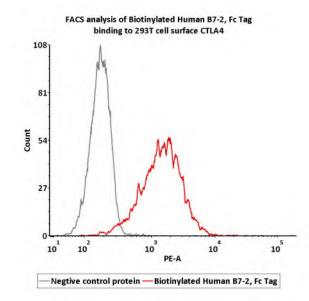
# **Bioactivity-FACS**



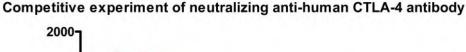
Flow Cytometry assay shows that Biotinylated Human B7-2, Fc,Avitag, premium grade (Cat. No. CD6-H82F5) can bind to CD28 expressed on Jurkat E6.1 cells. The concentration of B7-2 used is 1.5 μg/mL (Routinely tested).

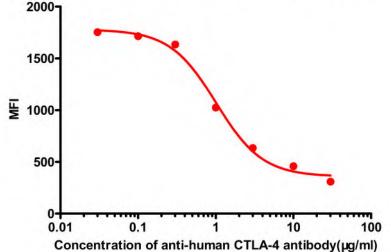


FACS analysis shows that the binding of Biotinylated Human B7-2, Fc,Avitag, premium grade (Cat. No. CD6-H82F5) to CD28 expressed on Jurkat E6.1 was inhibited by increasing concentration of neutralizing Anti-CD28 antibody. The concentration of B7-2 used is 1.5  $\mu$ g/mL. The IC50 is 0.031  $\mu$ g/mL (Routinely tested).



Flow Cytometry assay shows that Biotinylated Human B7-2, Fc, Avitag, premium grade (Cat. No. CD6-H82F5) can bind to 293 cell overexpressing





FACS analysis shows that the binding of Biotinylated Human B7-2, Fc, Avitag, premium grade (Cat. No. CD6-H82F5) to 293 overexpressing CTLA-4 was



## Biotinylated Human B7-2 / CD86 Protein, Fc,Avitag™, premium grade

Catalog # CD6-H82F5

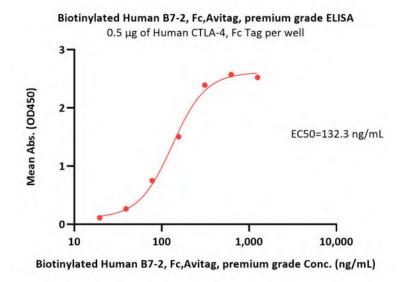


10

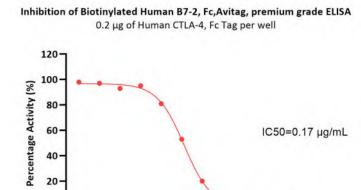
human CTLA-4. The concentration of Human B7-2 is 2  $\mu$ g/mL (Routinely tested).

inhibited by increasing concentration of neutralizing Anti-human CTLA-4 antibody. The concentration of Human B7-2 is 2  $\mu$ g/mL. The IC50 is 1.014  $\mu$ g/mL (Routinely tested).

## **Bioactivity-ELISA**



Immobilized Human CTLA-4, Fc Tag (Cat. No. CT4-H5255) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Biotinylated Human B7-2, Fc,Avitag, premium grade (Cat. No. CD6-H82F5) with a linear range of 19-156 ng/mL (QC tested).



Serial dilutions of Ipilimumab were added into Human CTLA-4, Fc Tag (Cat. No. CT4-H5255): Biotinylated Human B7-2, Fc, Avitag, premium grade (Cat. No. CD6-H82F5) binding reactions. The half maximal inhibitory concentration (IC50) is  $0.1701 \, \mu g/mL$  (Routinely tested).

0.1

Ipilimumab Conc. (μg/mL)

0.01

### **Background**

Cluster of Differentiation 86 (CD86) is also known as B-lymphocyte activation antigen B7-2, is a type I membrane protein that is a member of the immunoglobulin superfamily, and is constitutively expressed on interdigitating dendritic cells, Langerhans cells, peripheral blood dendritic cells, memory B cells, and germinal center B cells. Additionally, B72 is expressed at low levels on monocytes and can be upregulated through interferon γ. CD86 is the ligand for two different proteins on the T cell surface: CD28 (for autoregulation and intercellular association) and CTLA-4 (for attenuation of regulation and cellular disassociation). CD86 works in tandem with CD80 to prime T cells. Recent study has revealed that B7-2 promotes the generation of a mature APC repertoire and promotes APC function and survival. Furthermore, the B7 proteins are also involved in innate immune responses by activating NF-κB-signaling pathway in macrophages. CD86 thus is regarded as a promising candidate for immune therapy. CD86+ macrophages in Hodgkin lymphoma patients are an independent marker for potential nonresponse to firstline-therapy.

