

## Synonym

CD80,B7,B7-1,B7.1,BB1,CD28LG,CD28LG1,LAB7

#### **Source**

Human B7-1, Mouse IgG2a Fc Tag(B71-H52A4) is expressed from human 293 cells (HEK293). It contains AA Val 35 - Asn 242 (Accession # P33681-1). Predicted N-terminus: Val 35

### **Molecular Characterization**

B7-1(Val 35 - Asn 242) mFc(Glu 98 - Lys 330) P33681-1 P01863

This protein carries a mouse IgG2a Fc tag at the C-terminus.

The protein has a calculated MW of 50.9 kDa. The protein migrates as 65-85 kDa when calibrated against <u>Star Ribbon Pre-stained Protein Marker</u> under reducing (R) condition (SDS-PAGE) due to glycosylation.

#### **Endotoxin**

Less than 0.1 EU per µg by the LAL method / rFC method.

## **Purity**

>95% as determined by SDS-PAGE.

>90% as determined by SEC-MALS.

#### **Formulation**

Lyophilized from 0.22 µm filtered solution in

Tris with Glycine, Arginine and 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

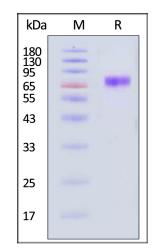
For long term storage, the product should be stored at lyophilized state at -20°C or lower.

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.

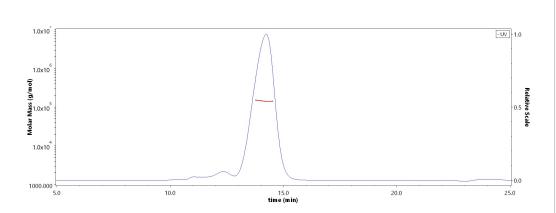
#### **SDS-PAGE**



Human B7-1, Mouse IgG2a Fc Tag on SDS-PAGE under reducing (R) condition. The gel was stained with Coomassie Blue. The purity of the protein is greater than 95% (With <u>Star Ribbon Pre-stained Protein Marker</u>).

#### **Bioactivity-ELISA**

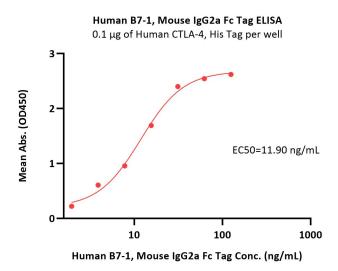
#### **SEC-MALS**

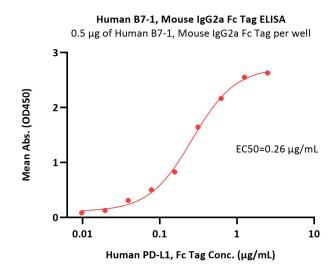


The purity of Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) is more than 90% and the molecular weight of this protein is around 135-155 kDa verified by SEC-MALS.

Report



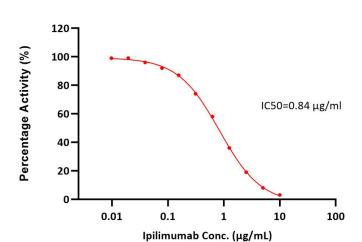


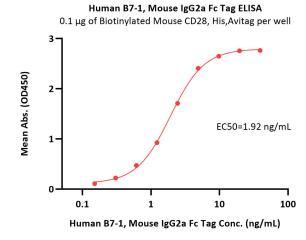


Immobilized Human CTLA-4, His Tag (Cat. No. CT4-H5229) at 1  $\mu$ g/mL (100  $\mu$ L/well) can bind Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) with a linear range of 2-31 ng/mL (QC tested).

Inhibition of Biotinylated Human CTLA-4, Fc, Avitag ELISA

Immobilized Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Human PD-L1, Fc Tag (Cat. No. PD1-H5258) with a linear range of 0.01-0.313  $\mu$ g/mL (Routinely tested).

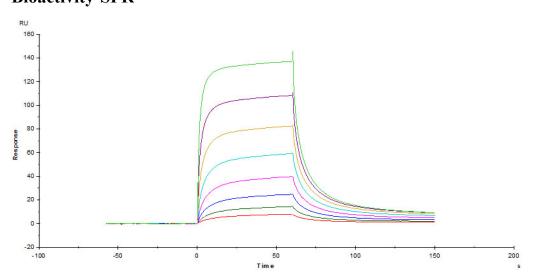




Serial dilutions of Ipilimumab were added into Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4): Biotinylated Human CTLA-4, Fc,Avitag (Cat. No. CT4-H82F3) binding reactions. The half maximal inhibitory concentration (IC50) is  $0.8402 \, \mu g/mL$  (Routinely tested).

Immobilized Biotinylated Mouse CD28, His,Avitag (Cat. No. CD8-M82E3) at 1  $\mu$ g/mL (100  $\mu$ L/well) on Streptavidin (Cat. No. STN-N5116) precoated (0.5  $\mu$ g/well) plate, can bind Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) with a linear range of 0.2-5 ng/mL (Routinely tested).

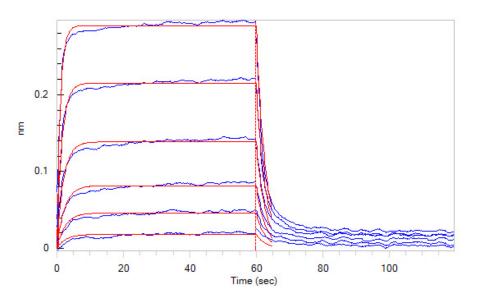
# **Bioactivity-SPR**



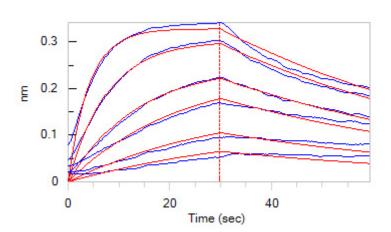
Immobilized Human / Cynomolgus / Rhesus macaque CD28, Fc Tag (Cat. No. CD8-H525a) on CM5 Chip can bind Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) with an affinity constant of 2.72  $\mu$ M as determined in a SPR assay (Biacore T200) (Routinely tested).



## **Bioactivity-BLI**

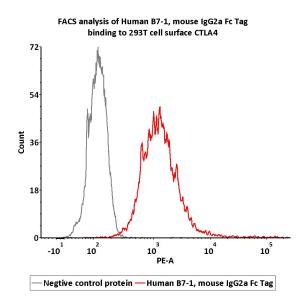


Loaded Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) on Protein A Biosensor, can bind Human / Cynomolgus / Rhesus macaque CD28, His Tag with an affinity constant of 4.6  $\mu$ M as determined in BLI assay (ForteBio Octet Red96e) (Routinely tested).



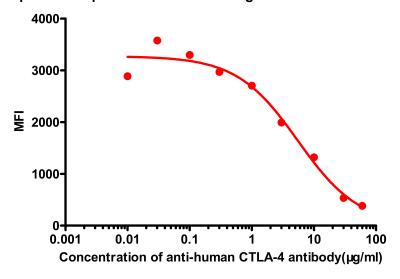
Loaded Biotinylated Human / Cynomolgus / Rhesus macaque CD28, Fc,Avitag (Cat. No. CD8-H82F2) on SA Biosensor, can bind Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) with an affinity constant of 38.3 nM as determined in BLI assay (ForteBio Octet Red96e) (Routinely tested).

## **Bioactivity-FACS**



Flow Cytometry assay shows that Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) can bind to 293 cell overexpressing human CTLA-4. The concentration of Human B7-1 is 1  $\mu$ g/mL (Routinely tested).

# Competitive experiment of neutralizing anti-human CTLA-4 antibody



FACS analysis shows that the binding of Human B7-1, Mouse IgG2a Fc Tag (Cat. No. B71-H52A4) to 293 overexpressing CTLA-4 was inhibited by increasing concentration of neutralizing Anti-human CTLA-4 antibody. The concentration of Human B7-1 is 1  $\mu$ g /mL. The IC50 is 5.5  $\mu$ g/mL (Routinely tested).

# Background

B7-1 and B7-2, together with their receptors CD28 and CTLA4, constitute one of the dominant co-stimulatory pathways that regulate T and Bcell responses. Although both CTLA4 and CD28 can bind to the same ligands, CTLA4 binds to B71 and B72 with a 20 100 fold higher affinity than CD28 and is involved in the downregulation of the immune response.

B-lymphocyte activation antigen B7-1 (referred to as B7) also known as cluster of Differentiation 80 (CD80), is a member of cell surface immunoglobulin superfamily and is expressed on activated B cells, activated T cells, macrophages and dendritic cells. It 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). CD80 works in tandem with CD86 to



# Human B7-1 / CD80 Protein, Mouse IgG2a Fc Tag, low endotoxin (MALS verified)





prime T cells. CD80 plays a role in induction of innate immune responses by activating NF-κB-signaling pathway in macrophages. CD80 is thus regarded as promising therapeutic targets for autoimmune diseases and various carcinomas.

