

### 21-1002: Human CD80 Recombinant Fc fusion Protein (Active)

Application : Functional Assay, ELISA, FACS, WB

Alternative Name : T-lymphocyte activation antigen CD80, Activation B7-1 antigen, BB1, CTLA-4 counter-receptor B7.1, B7, CD28LG, CD28LG1, LAB7

#### Description

The B-lymphocyte activation antigen B7-1 (referred to as B7), also known as CD80, is a member of cell surface immunoglobulin superfamily and is expressed on the surface of antigen-presenting cells including activated B cells, macrophages and dendritic cells. As costimulatory ligands, B7-1 which exists predominantly as dimer and the related protein B7-2, interact with the costimulatory receptors CD28 and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) expressed on T cells, and thus constitute one of the dominant pathways that regulate T cell activation and tolerance, cytokine production, and the generation of CTL. The B7/CD28/CTLA4 pathway has the ability to both positively and negatively regulate immune responses. CD80 is thus regarded as promising therapeutic targets for autoimmune diseases and various carcinomas. Cancer Immunotherapy Co- inhibitory Immune Checkpoint Targets Immune Checkpoint Detection

Human extracellular domain CD80 (B7-1) Fc fusion protein. This protein is expressed in CHO-K1 cells and purified using protein G colomn. Protein MW 53 kDa but SDS it runs arround 65 kDa due to glycosylation.

| Product Info               |   |
|----------------------------|---|
| Amount :<br>Purification : | 25 μg / 100 μg<br>95% Purity SDS-PAGE and HPLC  |
| Content :                  | Lyophilized from sterile PBS, 5% trehalose and 0.01% tween 80 are added as protectant before lyophilization. Reconstitutes sterile water.   |
| Storage condition :        | Store it under sterile conditions at -20°C to -80°C. It is recommended that the protein be aliquoted for optimal storage. Avoid repeated freeze-thaw cycles.  |
| Amino Acid :               | Human CD80 (ECD): MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSGVIHVTKEVKE<br>VATLSCGHNVSVEELAQTRIYWQKEKKMVLTMMSGDMNIWPEYK<br>NRTIFDITNNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLA<br>EVTLSVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEPHLSWL<br>ENGEELNAINTTVSQDPETELYAVSSKLDFNMTTNHSFMCLIKY GHLRVNQTFNWNTTKQEHFPDN |

### **Application Note**

Measured by its binding ability in a functional FLOW assay. Binding assay was tested using CHO-K1/CTLA4 cell line (cat no. 14-506ACL).

Endotoxin: <1.0EU per ug of the protein as determin by the LAL method.

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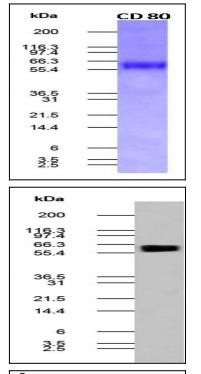


Figure-1: Human CD80/hFc recombinant protein. 0.5 ug protein was run on a 4-20% SDS-PAGE gel followed by Coomassie blue staining.

Figure-2: Western blot analysis of CD80/hFc recombinant protein (0.5ug) using antihuman CD80 antibody (Cat. No. 10-4108).

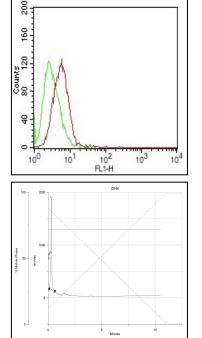


Figure-3: Binding activity of CD80/hFc recombinant protein to CTLA4 was analyzed by flow cytometry. 0.2 ug of CD80/hFc recombinant protein was incubated with CTLA4/CHO-K1 stable cells (Cat. No. 14-506ACL) or with parental CHO-K1 cells at 1 x 10^6 cells/reaction on ice for 1 h. Cells were washed once and then further incubated with FITC conjugated goat anti-hFc antibody on ice for 30 min. Cells were washed and then analyzed by flow cytometry. CTLA4/CHO-K1 stable cells (Red); Parental CHO-K1 cells (Green).

Figure-4: HPLC analysis of CD80-Fc recombinant protein.

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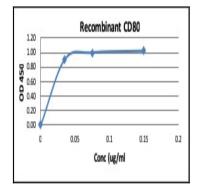


Figure 5: Binding activity of CD80/hFc recombinant protein to CTLA4 / CHO stable cell line (14-506ACL) was analyzed by cell based ELISA. CTLA4 / CHO cells were plated into 96 well ELISA plate over night. Next day, plate was washed in PBS and fixed in fixation buffer (Abeomics) for 15 min. Cells were blocked in blocking reagent (Abeomics) for 30 min. Then PD-1/hFc recombinant protein was added in different dilution to cells and incubated in room temp. for 1 hr. Plate was wash 3 times in TBST wash buffer. Goat anti-human HRP was added and incubated at room temp. for 30 min. Then plate was washed 4 times in TBST and analyzed in ELISA reader in 450 nm.