Validation data for hCTLA4-Fc

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Version 23L18-NJ

hCTLA-Fc is a soluble human CTLA-4 chimera protein generated by fusing the N-terminal extracellular domain of human CTLA-4 to the N-terminus of a human IgG1 Fc domain with a TEV (Tobacco Etch Virus) sequence linker. hCTLA-4-Fc has an apparent molecular weight of ~47 kDa on an SDS-PAGE gel **(Figure 1)**. It has been functionally validated by the detection of cell surface hCD80/86 using flow cytometry **(Figure 2)**, binding of an Anti-hCTLA-4 monoclonal antibody (mAb) using ELISA **(Figure 3)**, as well as inhibition of NFAT activation in Jurkat-Lucia™ TCR-hPD-1 reporter cells **(Figure 4)**.

hCTLA4-Fc analysis by SDS-PAGE

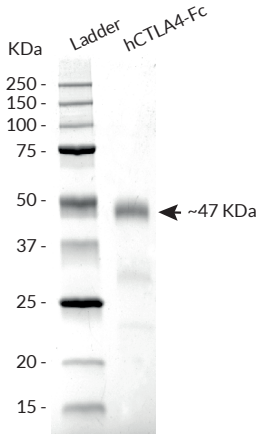

Figure 1: SDS-PAGE analysis of the hCTLA4-Fc protein. 0.5 µg of the fusion protein was loaded on a 12% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Bio-Rad). Detection was performed as per the manufacturer's instructions.

Cell surface staining using hCTLA4-Fc

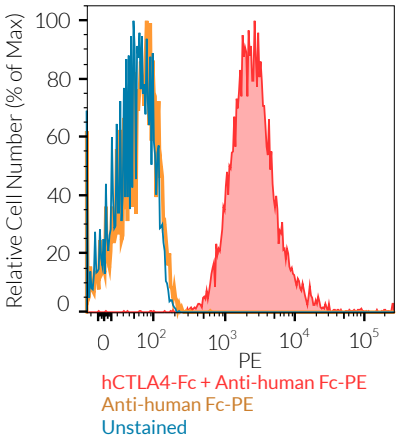

Figure 2: Human CD80/86 cell surface detection using hCTLA4-Fc. ~5 x 10^5 Raji-APC hPD-L1 cells were incubated with 2 µg of hCTLA4-Fc for 30 min at 4°C. Cells were then washed and incubated with 1 µl of mouse anti-human IgG Fc antibody coupled to PE for 30 min at 4°C. Cell surface staining was analyzed by flow cytometry.

ELISA detection of hCTLA4-Fc

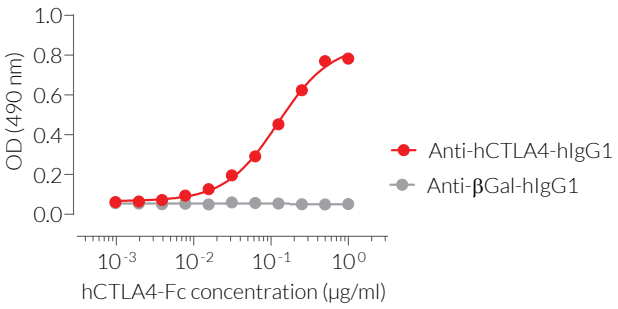

Figure 3: ELISA detection of hCTLA4-Fc with Anti-hCTLA4 mAb. A 2-fold serial dilution of the hCTLA4-Fc fusion protein was performed and coated on ELISA plates overnight. Anti-hCTLA4-hIgG1 (red curve) or Anti-βGal-hIgG1 control mAb (grey curve) at 5 µg/ml was added for the capture step. An HRP-labeled anti-human κ light chain antibody (1/1000 dilution) and the HRP substrate OPD (o-phenylenediamine dihydrochloride) were used for the detection step. Absorbance was read at 490 nm.

Activation blockade of Jurkat-Lucia™ TCR-hPD-1 cells

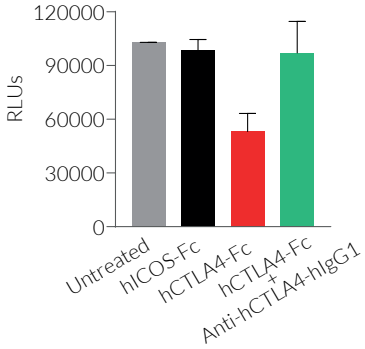

Figure 4: Activation of Jurkat-Lucia™ TCR-hPD-1 cells. ~2.5 x 10^5 Raji-APC hPD-L1 cells were incubated with hCTLA4-Fc (50 µg/ml) or hICOS-Fc control for 1 h at 37°C. 10^5 Jurkat-Lucia™ TCR-hPD-1 cells were then added either alone or with Anti-hCTLA4-hIgG1 (10 µg/ml). After 6 hours of incubation at 37°C, the NFAT activation in the Jurkat-Lucia™ TCR-hPD-1 cells was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia. Responses are shown as relative light units (RLUs; mean + SEM.)

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