Validation data for hICOS-Fc

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Version 23L07-NJ

hICOS-Fc is a soluble human ICOS chimera protein generated by fusing the N-terminal extracellular domain of human ICOS to the N-terminus of a human IgG1 Fc domain with a TEV (Tobacco Etch Virus) sequence linker. hICOS-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 hICOS-L using flow cytometry (**Figure 2**) and by binding of an Anti-hICOS monoclonal antibody (mAb) using ELISA (**Figure 3**).

hICOS-Fc analysis by SDS-PAGE

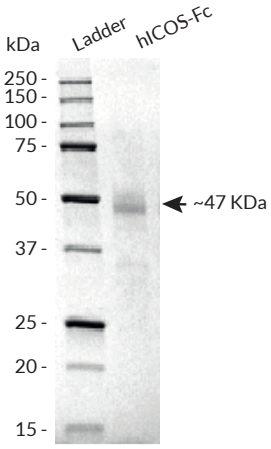

Figure 1: SDS-PAGE analysis of the hICOS-Fc protein. 0.5 µg of the fusion protein was loaded on a 12% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Bio-Rad). Detection was performed as per the manufacturer's instructions.

Cell surface staining using hICOS-Fc

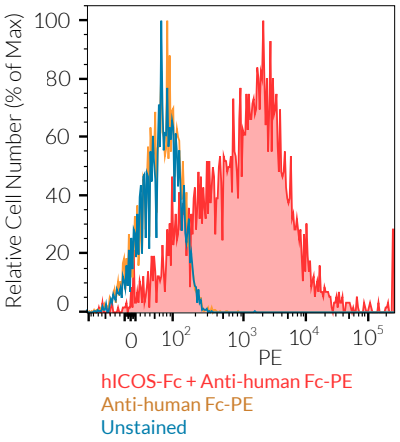

Figure 2: Human ICOS-L cell surface detection using hICOS-Fc. ~5 x 10^5 Raji-hICOS-L cells were incubated with 1 µg of hICOS-Fc for 30 min at 4°C. Cells were then washed and incubated with 1 µl of mouse anti-human IgG Fc antibody coupled to PE for 30 min at 4°C. Cell surface staining was analyzed by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

ELISA detection of hICOS-Fc

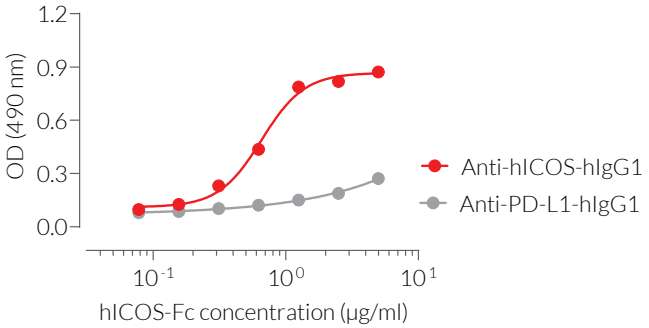

Figure 3: ELISA detection of hICOS-Fc with Anti-hICOS mAb. A 2-fold serial dilution of hICOS-Fc fusion protein was performed and coated on ELISA plates overnight. Anti-hICOS-hIgG1 (red curve) or Anti-PD-L1-hIgG1 control mAb (grey curve) at 2 µg/ml was added for the capture step. An HRP-labeled anti-human 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.

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