Validation data for hICOS-L-Fc

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Version 24D04-NJ

hICOS-L-Fc is a soluble human ICOS-L chimera protein generated by fusing the N-terminal extracellular domain of human ICOS-L to the N-terminus of a human IgG1 Fc domain with a TEV (Tobacco Etch Virus) sequence linker. hICOS-L-Fc has an apparent molecular weight of ~70 kDa on an SDS-PAGE gel **(Figure 1)**. It has been functionally validated by the detection of cell surface hICOS using flow cytometry **(Figure 2)**, binding of an Anti-hICOS-L monoclonal antibody (mAb) using ELISA **(Figure 3)**, as well as NFAT activation in Jurkat-Lucia™-hICOS reporter cells **(Figure 4)**.

hICOS-L-Fc analysis by SDS-PAGE

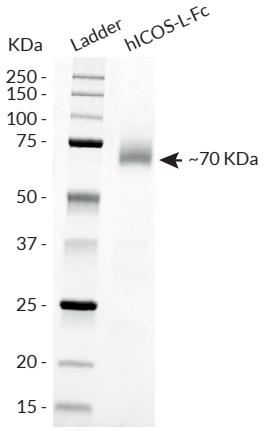

Figure 1: SDS-PAGE analysis of the hICOS-L-Fc protein. 0.5 µg of the fusion protein was loaded on a 12% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Bio-Rad). Detection was performed as per the manufacturer's instructions.

Cell surface staining using hICOS-L-Fc

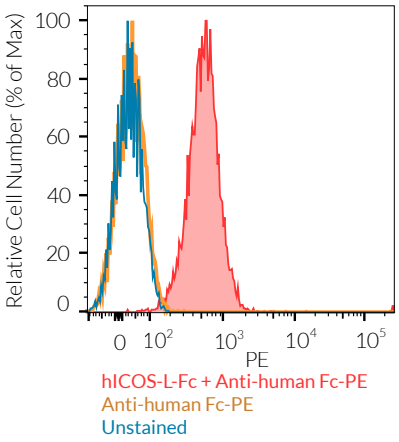

Figure 2: Human ICOS cell surface detection using hICOS-L-Fc. ~5 x 10^5 Jurkat-Lucia™ hICOS cells were incubated with 500 ng of hICOS-L-Fc for 30 min at 4°C. Cells were then washed and incubated with 1 µl of mouse anti-human IgG Fc antibody coupled to PE for 30 min at 4°C. Cell surface staining was analyzed by flow cytometry.

ELISA detection of hICOS-L-Fc

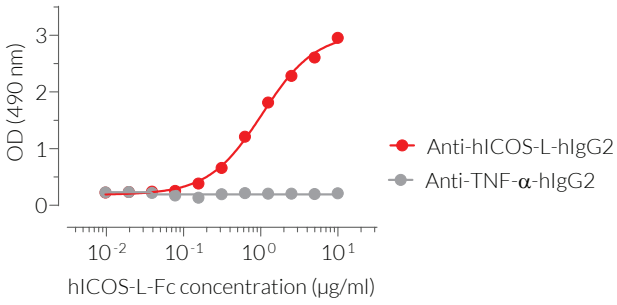

Figure 3: ELISA detection of hICOS-L-Fc with Anti-hICOS-L mAb. A 2-fold serial dilution of the hICOS-L-Fc fusion protein was performed and coated on ELISA plates overnight. Anti-hICOS-L-hIgG2 (red curve) or Anti-TNFα-hIgG2 control mAb (grey curve) at 5 µg/ml was added for the capture step. An HRP-labeled 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 of Jurkat-Lucia™-hICOS cells

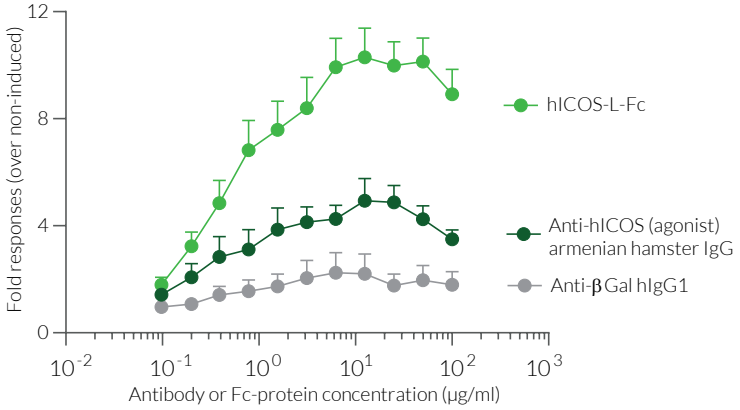

Figure 4: Activation of Jurkat-Lucia™-hICOS cells. Jurkat-Lucia™-hICOS cells were incubated with increasing concentrations of recombinant hICOS-L-Fc fusion protein, agonist Anti-hICOS IgG, or control Anti-βGal hIgG1 mAbs for 6 hours. NFAT activation in the Jurkat-Lucia™-hICOS cells was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Fold responses are shown as mean + SEM.

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