Validation data for Fc-hCD70

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Version 24D29-NJ

Fc-hCD70 is a soluble human CD70 chimera protein generated by fusing the N-terminal extracellular domain of human CD70 to the C-terminus of a human IgG1 Fc domain with a TEV (Tobacco Etch Virus) sequence linker. Fc-hCD70 has an apparent molecular weight of ~48 kDa on an SDS-PAGE gel **(Figure 1)**. It has been functionally validated by the detection of cell surface hCD27 using flow cytometry **(Figure 2),** binding of an anti-hCD70 monoclonal antibody (mAb) using ELISA **(Figure 3)**, as well as NF-κB activation in Jurkat-Lucia™ hCD27 reporter cells **(Figure 4)**.

Fc-hCD70 analysis by SDS-PAGE

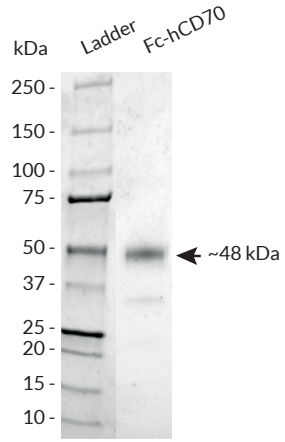

Figure 1: SDS-PAGE analysis of the Fc-hCD70 protein. 0.5 µg of the fusion protein was loaded on a 12% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Bio-Rad). Detection was performed as per the manufacturer's instructions.

Cell surface staining using Fc-hCD70

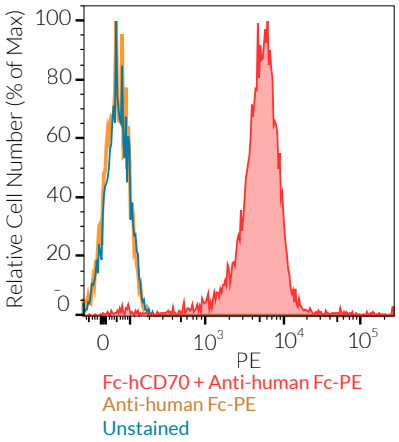

Figure 2: Human CD27 cell surface detection using Fc-hCD70. ~5 x 10^5 Jurkat-Lucia™ hCD27 cells were incubated with 2 µg of Fc-hCD70 for 30 min at 4°C. Cells were then washed and incubated with 1 µl of mouse anti-human IgG Fc antibody coupled to PE for 30 min at 4°C. Cell surface staining was analyzed by flow cytometry.

ELISA detection of Fc-hCD70

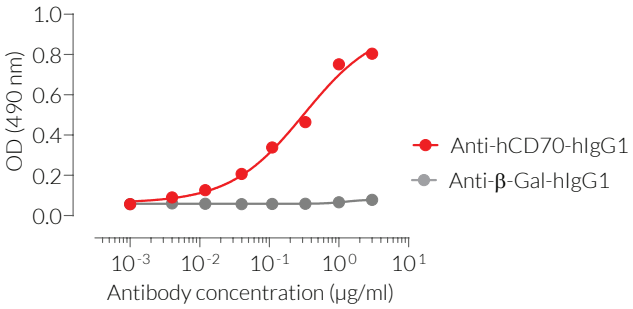

Figure 3: ELISA detection of Fc-hCD70 with Anti-hCD70-hIgG1 mAb. The Fc-hCD70 fusion protein was performed and coated at 2 µg/ml on ELISA plates overnight. A 3-fold serial dilution of Anti-hCD70-hIgG1 (red curve) or Anti-β-Gal-hIgG1 control mAb (grey curve) was added for the capture step. An HRP-labeled 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™ hCD27 cells

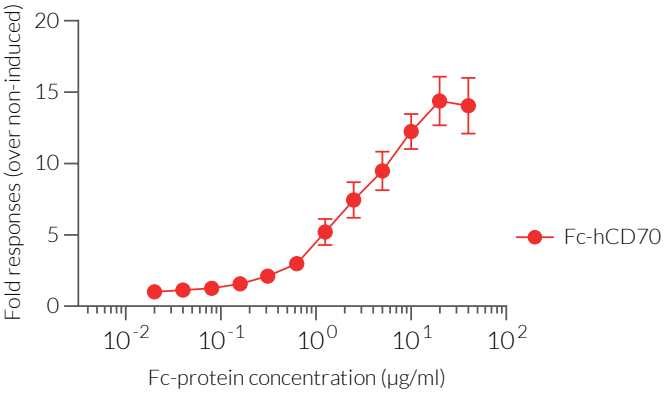

Figure 4: Activation of Jurkat-Lucia™ hCD27 cells. Jurkat-Lucia™ hCD27 cells were incubated with increasing concentrations of recombinant human Fc-CD70 fusion protein for 24 hours. The NF-κB activation in Jurkat-Lucia™ hCD27 cells was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ 4. Fold responses are shown as mean ± SEM.

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