Validation data for hCD27-Fc

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Version 24D23-NJ

hCD27-Fc is a soluble human CD27 chimera protein generated by fusing the N-terminal extracellular domain of human CD27 to the N-terminus of a human IgG1 Fc domain with a TEV (Tobacco Etch Virus) sequence linker. hCD27-Fc has an apparent molecular weight of ~55 kDa on an SDS-PAGE gel **(Figure 1)**. It has been functionally validated by the detection of cell surface hCD70 using flow cytometry **(Figure 2)** and binding of an anti-hCD27 monoclonal antibody (mAb) using ELISA **(Figure 3)**.

hCD27-Fc analysis by SDS-PAGE

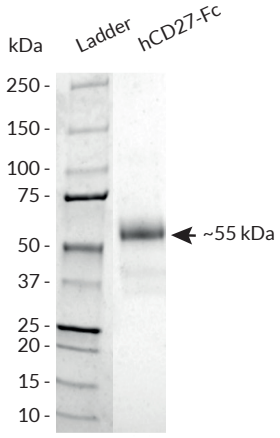

Figure 1: SDS-PAGE analysis of the hCD27-Fc protein. 0.5 µg of the fusion protein was loaded on a 12% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Bio-Rad). Detection was performed as per the manufacturer's instructions.

Cell surface staining using hCD27-Fc

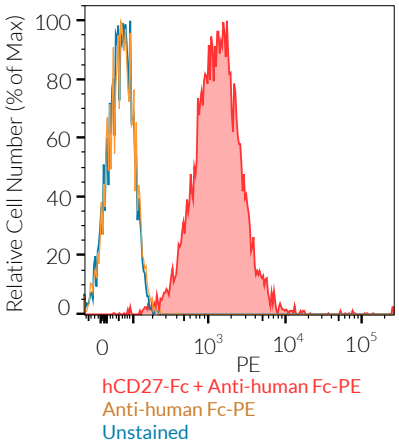

Figure 2: Human CD70 cell surface detection using hCD27-Fc. ~5 x 10^5 Raji-APC-Null cells were incubated with 2 µg of hCD27-Fc for 30 min at 4°C. Cells were then washed and incubated with 1 µl of mouse anti-human IgG Fc antibody coupled to PE for 30 min at 4°C. Cell surface staining was analyzed by flow cytometry.

ELISA detection of hCD27-Fc

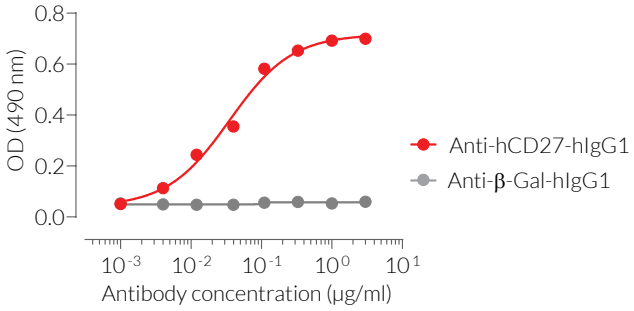

Figure 3: ELISA detection of hCD27-Fc with Anti-hCD27-hIgG1 mAb. The hCD27-Fc fusion protein was performed and coated at 2 µg/ml on ELISA plates overnight. A 3-fold serial dilution of Anti-hCD27-hIgG1 (red curve) or Anti-β-Gal-hIgG1 control mAb (grey curve) was added for the capture step. An HRP-labeled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

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