Validation data for Fc-h4-1BBL

https://www.invivogen.com/cd137l-4-1bbl-fc

For research use only

Version 24D29-NJ

Fc-h4-1BBL is a soluble human 4-1BBL chimera protein generated by fusing the N-terminal extracellular domain of human 4-1BBL to the C-terminus of a human IgG1 Fc domain with a TEV (Tobacco Etch Virus) sequence linker. Fc-h4-1BBL has an apparent molecular weight of ~50 kDa on an SDS-PAGE gel **(Figure 1)**. It has been functionally validated by the detection of cell surface h4-1BB using flow cytometry **(Figure 2),** binding of an anti-h4-1BBL monoclonal antibody (mAb) using ELISA **(Figure 3)**, as well as NF-κB activation in Jurkat-Lucia™ h4-1BB reporter cells **(Figure 4)**.

Fc-h4-1BBL analysis by SDS-PAGE

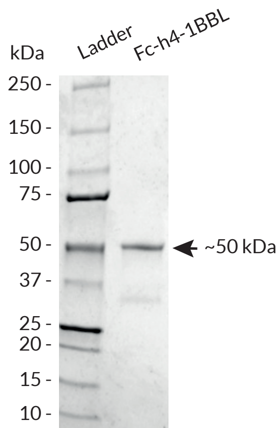

Figure 1: SDS-PAGE analysis of the Fc-h4-1BBL protein. 0.5 µg of the fusion protein was loaded on a 12% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Bio-Rad). Detection was performed as per the manufacturer's instructions.

Cell surface staining using Fc-h4-1BBL

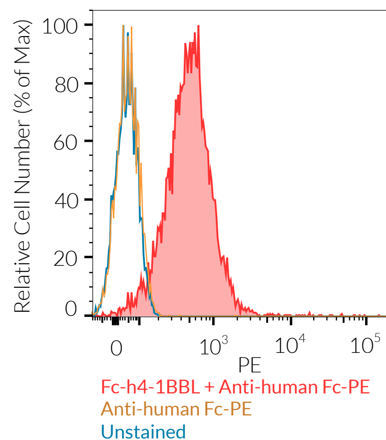

Figure 2: Human 4-1BB cell surface detection using Fc-h4-1BBL. ~5 x 10^5 Jurkat-Lucia™ h4-1BB cells were incubated with 2 µg of Fc-h4-1BBL for 30 min at 4°C. Cells were then washed and incubated with 1 µl of mouse anti-human IgG Fc antibody coupled to PE for 30 min at 4°C. Cell surface staining was analyzed by flow cytometry.

ELISA detection of Fc-h4-1BBL

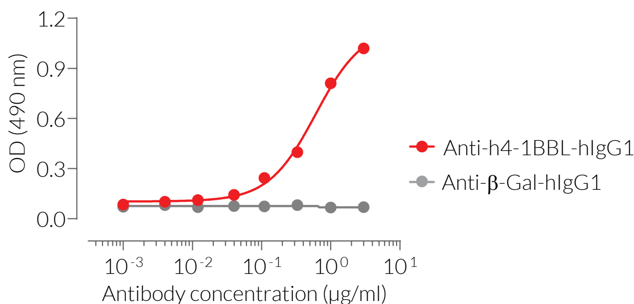

Figure 3: ELISA detection of Fc-h4-1BBL with Anti-h4-1BBL-hIgG1 mAb. The Fc-h4-1BBL fusion protein was performed and coated at 2 µg/ml on ELISA plates overnight. A 3-fold serial dilution of Anti-h4-1BBL-hIgG1 (red curve) or Anti-β-Gal-hIgG1 control mAb (grey curve) was added for the capture step. An 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™ h4-1BB cells

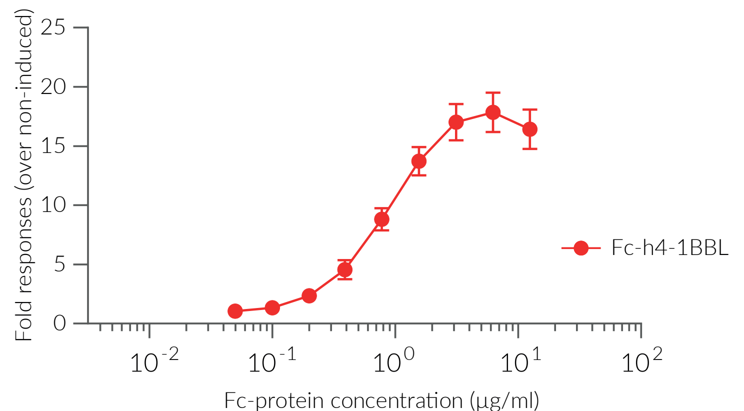

Figure 4: Activation of Jurkat-Lucia™ h4-1BB cells. Jurkat-Lucia™ h4-1BB cells were incubated with increasing concentrations of recombinant human Fc-4-1BBL fusion protein for 24 hours. The NF-κB activation in Jurkat-Lucia™ h4-1BB cells was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ 4. Fold responses are shown as mean ± SEM.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3-622-34-80
E-mail: info@invivogen.com