Validation data for hPD1-Fc

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Version 23L08-NJ

hPD1-Fc is a soluble human PD-1 chimera protein generated by fusing the N-terminal extracellular domain of human PD-1 to the N-terminus of a human IgG1 Fc domain with a TEV (Tobacco Etch Virus) sequence linker. hPD1-Fc has an apparent molecular weight of ~62 kDa on an SDS-PAGE gel **(Figure 1)**. It has been functionally validated by the detection of cell surface hPD-L1 using flow cytometry **(Figure 2)** and by binding of Anti-hPD-1 monoclonal antibodies (mAbs) using ELISA **(Figure 3)**.

hPD1-Fc analysis by SDS-PAGE

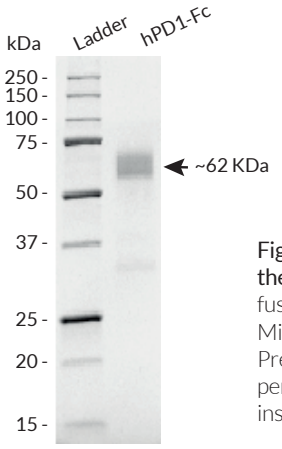

Figure 1: SDS-PAGE analysis of the hPD1-Fc protein. 0.5 µg of the fusion protein was loaded on a 12% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (Bio-Rad). Detection was performed as per the manufacturer's instructions.

Cell surface staining using hPD1-Fc

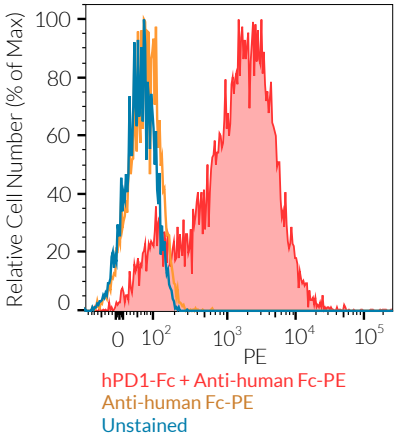

Figure 2: Human PD-L1 cell surface detection using hPD1-Fc. ~5 x 10^5 Raji-APC-hPD-L1 cells were incubated with 2 µg of hPD1-Fc for 45 min at 4°C. Cells were then washed and incubated with 1 µg of mouse anti-human IgG Fc antibody coupled to PE for 1h at 4°C. Cell surface staining was analyzed by flow cytometry.

ELISA detection of hPD1-Fc

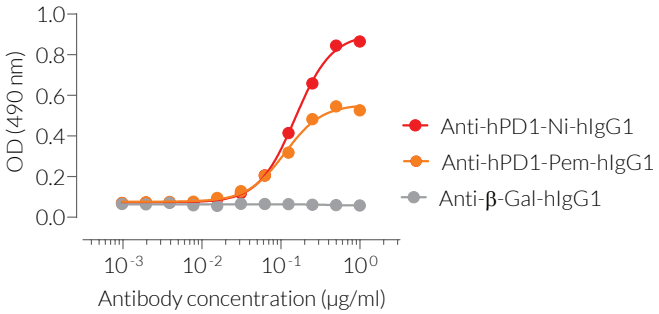

Figure 3: ELISA detection of hPD1-Fc with Anti-hPD1 mAbs. hPD1-Fc fusion protein (1 µg/ml) was coated on ELISA plates overnight. A 2-fold serial dilution of Anti-hPD1-hIgG1 Nivolumab biosimilar (red curve) or Pembrolizumab biosimilar (orange curve), or Anti-β-Gal-hIgG1 control mAb (grey curve) was performed 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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