

Validation data for Raji-hPD-L1 Cells

<https://www.invivogen.com/raji-hpd1>

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Version 20C16-ED

Raji-hPD-L1 cells were developed from the Raji cell line to overexpress the human programmed cell death ligand 1 (PD-L1, also known as CD274 or B7-H1) gene. Raji-hPD-L1 cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human PD-L1 monoclonal antibodies (mAbs). Human PD-L1 expression by Raji-hPD-L1 cells has been verified by flow-cytometry (**Figure 1**), and induction of ADCC has been validated using InvivoGen's combinations of anti-human PD-L1 antibody isotypes and Jurkat-NFAT Lucia™ CD16 reporter cell line (**Figure 2**). The level of ADCC induction is measured as a bioluminescent signal produced by an NFAT-dependent Lucia luciferase reporter protein. Antibodies displaying lower EC₅₀ have higher ADCC potency.

Validation of PD-L1 expression

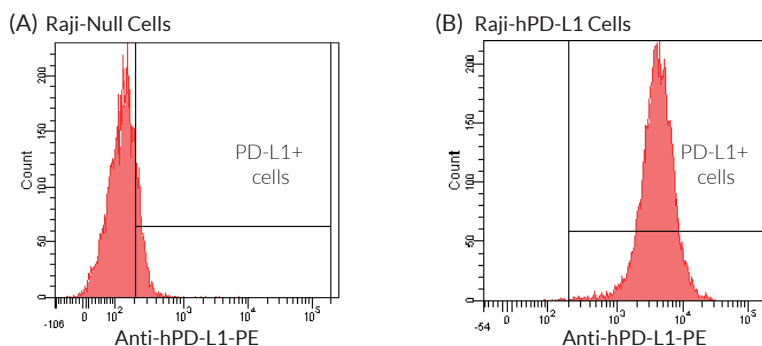


Figure 1: Validation of the expression of human PD-L1 by Raji-hPD-L1 cells. Raji-Null (A) and Raji-hPD-L1 (B) cells were incubated with a PE-conjugated Anti-hPD-L1 mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-human PD-L1 (Atezolizumab) antibody isotypes and Raji-hPD-L1 target cells

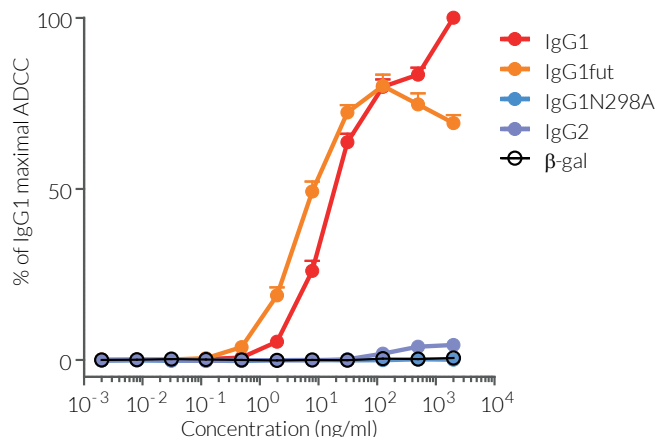


Figure 2: Comparison of ADCC potency for native and engineered anti-human PD-L1 antibody isotypes. Raji-hPD-L1 cells were incubated with gradient concentrations of Anti-hPD-L1 or Anti- β -galactosidase (β -gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with targets cells for 6 hours. NFAT activation, reflecting the induced ADCC response, was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Percentages of the maximal response normalized to the IgG1 isotype are shown.

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