

Validation data for Raji-hCTLA4 Cells

<https://www.invivogen.com/raji-hctla4>

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Version 20C13-ED

Raji-hCTLA4 cells were developed from the Raji cell line to overexpress the human cytotoxic T cell lymphocyte antigen-4 (CTLA-4, also known as CD152) gene. Raji-hCTLA4 cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human CTLA-4 monoclonal antibodies (mAbs). Human CTLA-4 expression by Raji-hCTLA4 cells has been verified by flow-cytometry (Figure 1), and induction of ADCC has been validated using InvivoGen's combinations of anti-human CTLA-4 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 CTLA-4 expression

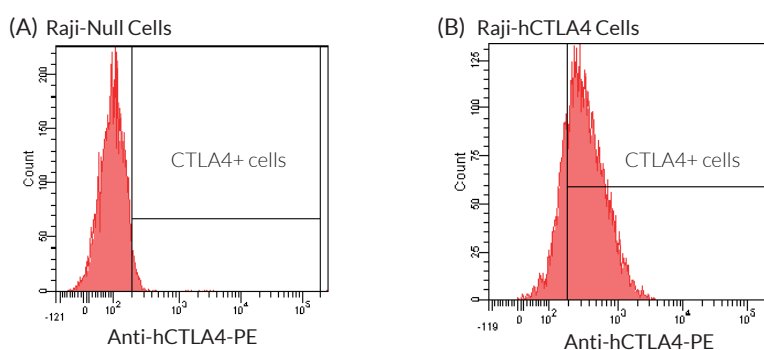


Figure 1: Validation of the expression of human CTLA-4 by Raji-hCTLA4 cells. Raji-Null (A) and Raji-hCTLA4 (B) cells were incubated with a PE-conjugated Anti-hCTLA4 mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-human CTLA-4 (Ipilimumab) antibody isotypes and Raji-hCTLA4 target cells

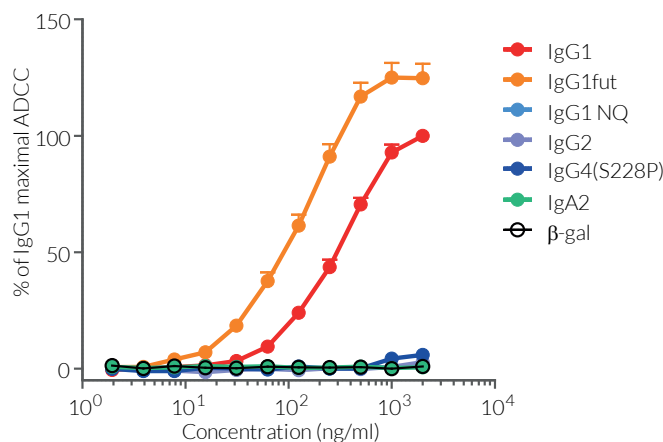


Figure 2: Comparison of ADCC potency for native and engineered anti-human CTLA-4 antibody isotypes. Raji-hCTLA4 cells were incubated with gradient concentrations of Anti-hCTLA4 or Anti-β-galactosidase (β-gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with targets 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.

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 Hong Kong: +852 3622-3480

E-mail: info@invivogen.com

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www.invivogen.com