

Validation data for Raji-hICOS Cells

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

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Version 20L15-ED

Raji-hICOS cells were developed from the Raji cell line to overexpress the human ICOS gene. Raji-hICOS cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human ICOS monoclonal antibodies (mAbs). Human ICOS expression by Raji-hICOS cells has been verified by flow-cytometry (**Figure 1**), and induction of ADCC has been validated using a collection of anti-human ICOS antibody isotypes and Jurkat-Lucia™ NFAT-CD16 reporter cells (**Figure 2**). The level of ADCC induction is measured by an NFAT-dependent Lucia luciferase reporter protein. Antibodies displaying lower EC₅₀ have higher ADCC potency.

Validation of ICOS expression by flow cytometry

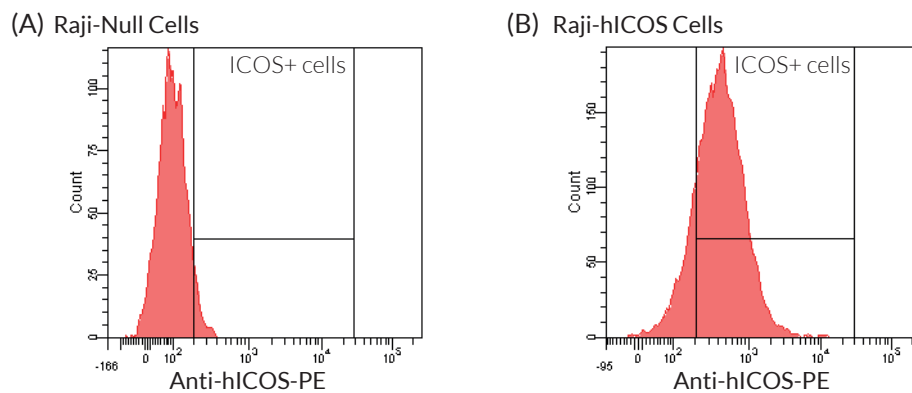


Figure 1: Validation of the expression of human ICOS by Raji-hICOS cells. Raji-Null (A) and Raji-hICOS (B) cells were incubated with a PE-conjugated Anti-hICOS mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-human ICOS antibody isotypes and Raji-hICOS target cells

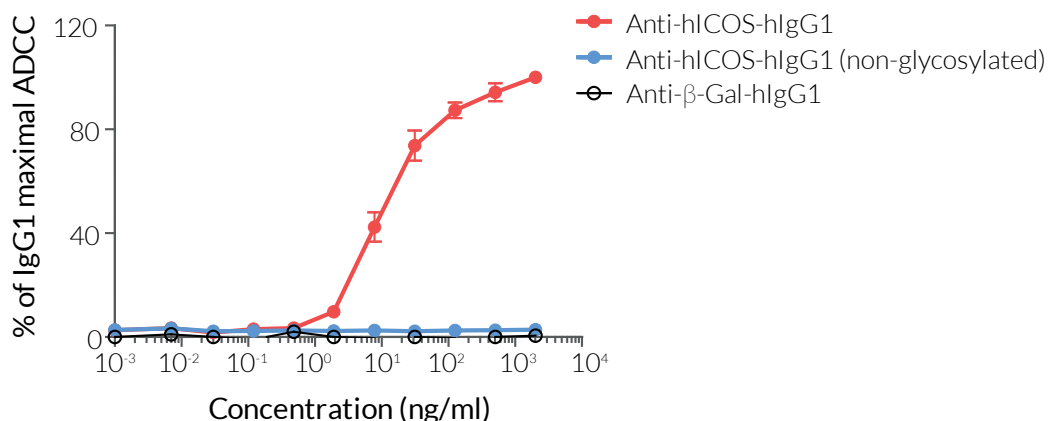


Figure 2: Comparison of ADCC potency for native and engineered anti-human ICOS antibody isotypes. Raji-hICOS cells were incubated with gradient concentrations of Anti-hICOS or Anti-β-galactosidase (β-Gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with target cells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

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