Validation data for Raji-hEGFR Cells

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Raji-hEGFR cells were developed from the Raji cell line to stably overexpress the human EGFR gene. Raji-hEGFR cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human EGFR monoclonal antibodies (mAbs). Human EGFR expression by Raji-hEGFR cells has been verified by flow-cytometry **(Figure 1)**, and induction of ADCC has been validated using InvivoGen's collection of anti-human EGFR 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.

Validation of EGFR expression by flow cytometry

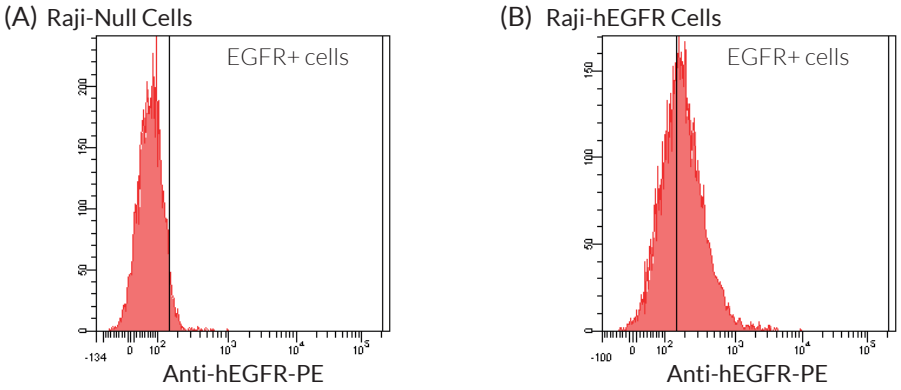

Figure 1: Validation of the expression of human EGFR by Raji-hEGFR cells. Raji-Null (A) and Raji-hEGFR (B) cells were incubated with a PE-conjugated Anti-hEGFR mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-human EGFR antibody isotypes and Raji-hEGFR target cells

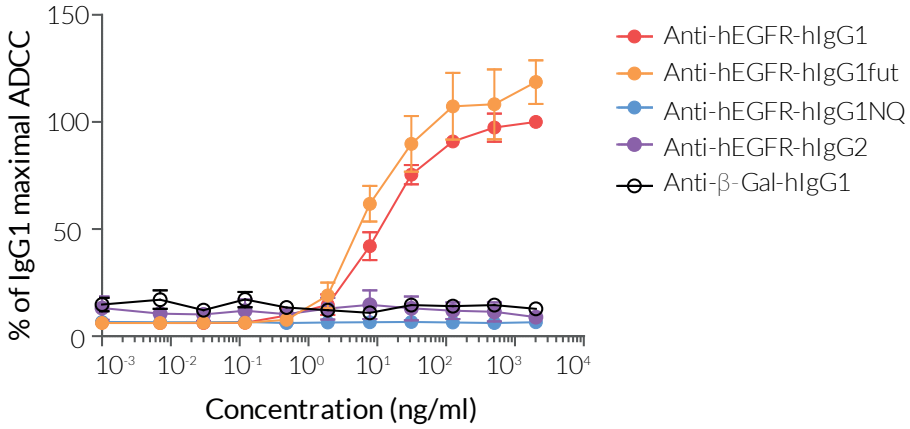

Figure 2: Comparison of ADCC potency for native and engineered anti-human EGFR antibody isotypes. Raji-hEGFR cells were incubated with gradient concentrations of Anti-hEGFR or Anti-β-galactosidase (β-Gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with target cells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