

Validation data for Raji-hVISTA Cells

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

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Version 20D15-ED

Raji-hVISTA cells were developed from the Raji cell line to overexpress the human VISTA gene. Raji-hVISTA cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human VISTA monoclonal antibodies (mAbs). Human VISTA expression by Raji-hVISTA cells has been verified by flow-cytometry (Figure 1), and induction of ADCC has been validated using InvivoGen's collection of anti-human VISTA antibody isotypes and Jurkat-Lucia™ NFAT-CD16 reporter cells (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 VISTA expression

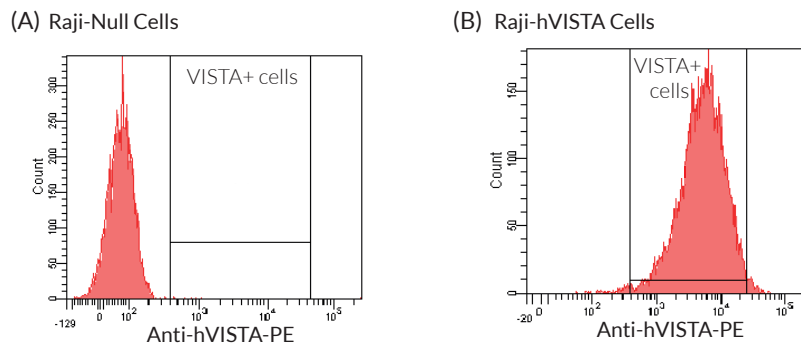


Figure 1: Validation of the expression of human VISTA by Raji-hVISTA cells. Raji-Null (A) and Raji-hVISTA (B) cells were incubated with a PE-conjugated Anti-hVISTA mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-human VISTA antibody isotypes and Raji-hVISTA target cells

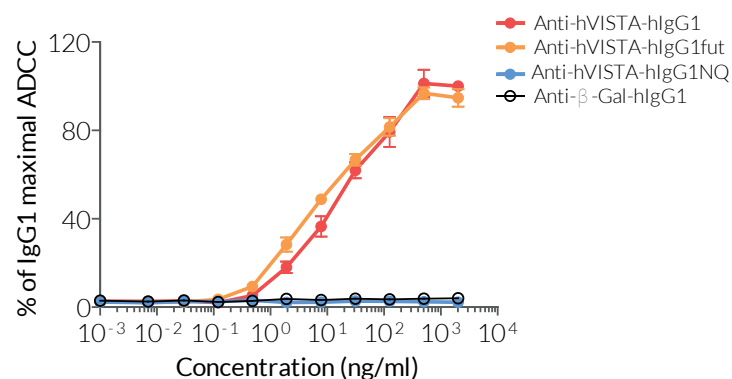


Figure 2: Comparison of ADCC potency for native and engineered anti-human VISTA antibody isotypes. Raji-hVISTA cells were incubated with gradient concentrations of Anti-hVISTA or Anti-β-galactosidase (β-gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with target cells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