

# Validation data for Raji-hPD-1 Cells

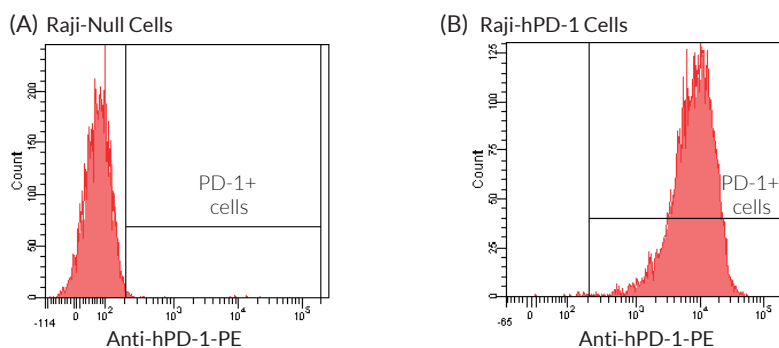
<https://www.invivogen.com/raji-hpd1>

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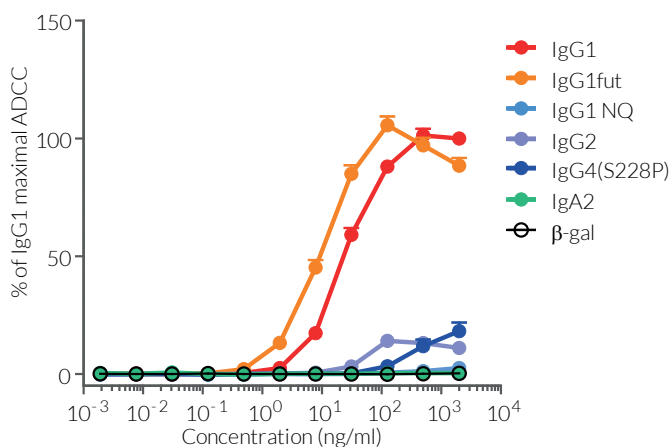
Raji-hPD-1 cells were developed from the Raji cell line to overexpress the human programmed cell death 1 (PD-1, also known as CD279) gene. Raji-hPD-1 cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human PD-1 monoclonal antibodies (mAbs). Human PD-1 expression by Raji-hPD-1 cells has been verified by flow-cytometry (**Figure 1**), and induction of ADCC has been validated using InvivoGen's combinations of anti-human PD-1 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 EC<sub>50</sub> have higher ADCC potency.

## Validation of PD-1 expression



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

## ADCC assay using various anti-human PD-1 (Nivolumab) antibody isotypes and Raji-hPD-1 target cells



**Figure 2:** Comparison of ADCC potency for native and engineered anti-human PD-1 antibody isotypes. Raji-hPD-1 cells were incubated with gradient concentrations of Anti-hPD-1 or Anti- $\beta$ -galactosidase ( $\beta$ -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

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