# Validation data for CTLA4/CD80 Bio-IC™

invivogen.com/immune-checkpoint-hctla4-hcd80-bioassay

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Version 25F12-NJ

CTLA4/CD80 Bio-IC™ is a bioluminescent cell-based assay designed for the screening of novel inhibitors of the CTLA4/CD80 immune checkpoint (IC) axis, such as monoclonal antibodies (mAbs), Fc-fusion proteins, or small molecule-based inhibitors. The assay relies on the co-culture of two cell lines:

**Jurkat-Lucia™ TCR-hCTLA4 effector cells** stably express a specific [HLA::peptide]-restricted TCR and an NFAT-inducible Lucia luciferase reporter gene. In addition, these cells overexpress the human (h)CD28 (cluster of differentiation 28) stimulatory receptor and the hCTLA4 (cytotoxic T lymphocyte-associated protein 4) inhibitory receptor **(Figure 1)**.
**Raji-APC-Null cells** stably express the specific [HLA::peptide]. They express endogenous hCD80, the inhibitory ligand for CTLA4 **(Figure 2)**.

This cellular assay has been functionally tested using the biosimilar Anti-hCTLA4 Ipilimumab. Upon addition of the blocking mAb, the CTLA4/CD80 inhibitory interaction is disrupted and Jurkat-Lucia™ TCR-hCTLA4 effector cells express Lucia luciferase in a dose-dependent manner **(Figure 3)**.

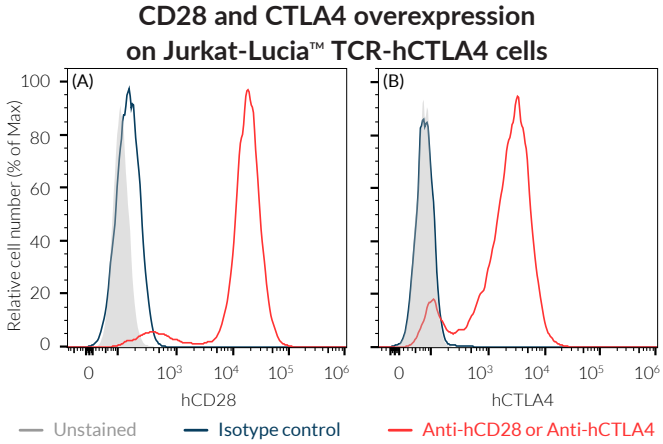


**Figure 1: Validation of human CD28 and CTLA4 overexpression by Jurkat-Lucia™ TCR-hCTLA4 cells.** Jurkat-Lucia™ TCR-hCTLA4 cells were incubated with a PE-conjugated Anti-hCD28 **(A)** or APC-conjugated Anti-hCTLA4 **(B)** mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

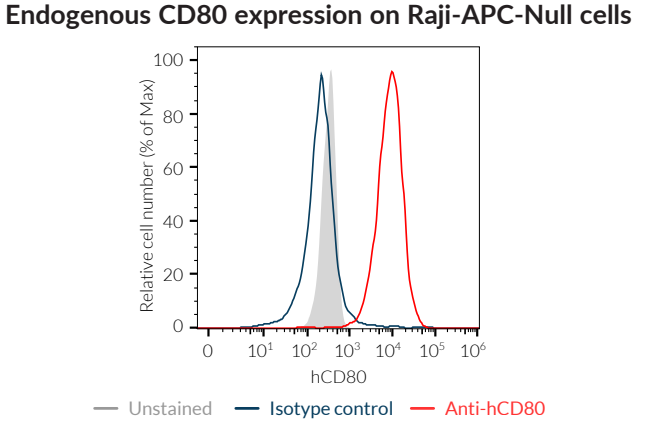


**Figure 2: Validation of endogenous human CD80 expression by Raji-APC-Null cells.** Raji-APC-Null cells were incubated with a PE-conjugated Anti-hCD80 mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

## Disruption of CTLA4/CD80 inhibitory interaction using Anti-hCTLA4 biosimilar antibody

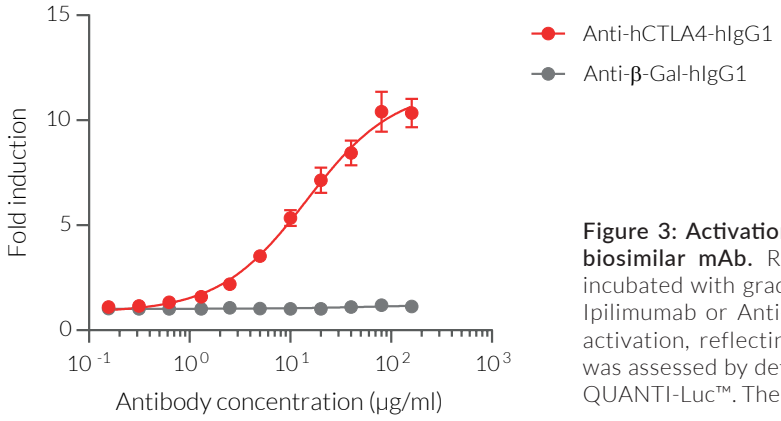


**Figure 3: Activation of Jurkat-Lucia™ TCR-hCTLA4 cells using Anti-hCTLA4 biosimilar mAb.** Raji-APC-Null and Jurkat-Lucia™ TCR-hCTLA4 cells were incubated with gradient concentrations of the biosimilar Anti-hCTLA4-hIgG1 Ipilimumab or Anti-β-Gal-hIgG1, as a negative control, for 24 hours. NFAT activation, reflecting the disruption of CTLA4/CD80 inhibitory interaction, was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. The fold increase over non induced cells (no mAbs) is shown.


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