Validation data for PD-1/PD-L1 Bio-IC™

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Version 23J17-NJ

PD-1/PD-L1 Bio-IC™ is a bioluminescent cell-based assay designed for the screening of novel inhibitors of the PD-1/PD-L1 immune checkpoint (IC) axis. The assay consists of two engineered cell lines. **Jurkat-Lucia™ TCR-hPD-1 effector cells** stably express a specific [HLA::peptide]-restricted TCR and an NFAT-inducible Lucia luciferase reporter gene. In addition, these cells overexpress human (h) CD28 stimulatory receptor **(Figure 1A)** and hPD-1 (programmed cell death 1) inhibitory receptor **(Figure 1B). Raji-APC-hPD-L1 cells** stably express the specific [HLA::peptide] and overexpress hPD-L1 (programmed cell death ligand 1) **(Figure 2)**. These cells have been functionally tested with various Anti-hPD-1 or Anti-hPD-L1 hIgG1 mAbs. Upon addition of blocking mAbs, the PD-1/PD-L1 inhibitory interaction is disrupted and Jurkat-Lucia™ TCR-hPD-1 effector cells express Lucia luciferase in a dose-dependent manner **(Figure 3)**.

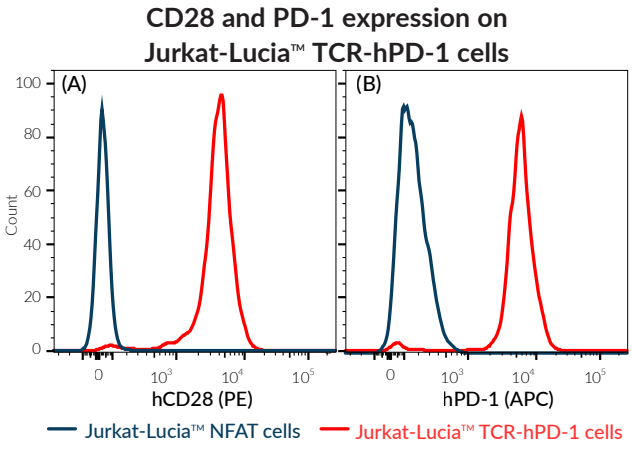

Figure 1: Validation of human CD28 and PD-1 expression by Jurkat-Lucia™ TCR-hPD-1 cells. Jurkat-Lucia™ NFAT and Jurkat-Lucia™ TCR-hPD-1 cells were incubated with a PE-conjugated Anti-hCD28 **(A)** or APC-conjugated Anti-hPD-1 **(B)** mAb for 30 minutes. The binding affinity was then measured using flow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

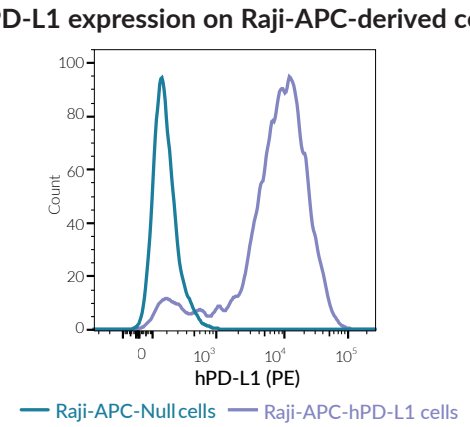

Figure 2: Validation of human PD-L1 expression by Raji-APC-hPD-L1 cells. Raji-APC-Null and Raji-APC-hPD-L1 cells were incubated with a PE-conjugated Anti-hPD-L1 mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

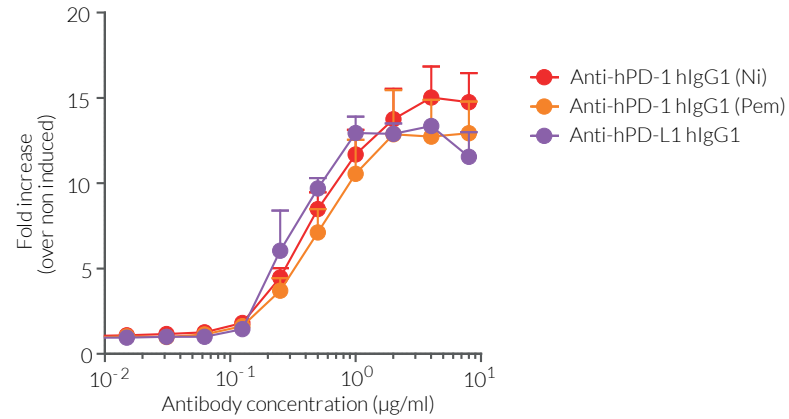

Figure 3: Activation of Jurkat-Lucia™ TCR-hPD-1 cells. Raji-APC-hPD-L1 and Jurkat-Lucia™ TCR-hPD-1 cells were incubated with gradient concentrations of Anti-hPD-1 hIgG1 (Ni: Nivolumab variable region; Pem: Pembrolizumab variable region) or Anti-hPD-L1 mAbs for 6 hours. NFAT activation, reflecting the disruption of PD-1/PD-L1 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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