Validation data for 4-1BB/4-1BBL Bio-IC™

https://www.invivogen.com/h41bb-bioassay

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Version 24D29-NJ

4-1BB/4-1BBL Bio-IC™ is a bioluminescent cell-based assay designed for the screening of novel inhibitors of the 4-1BB/4-1BBL immune checkpoint (IC) axis. The assay consists of two engineered cell lines. **Jurkat-Lucia™ h4-1BB effector cells** stably express human 4-1BB at the plasma membrane, as well as an NF-κB-inducible Lucia luciferase reporter gene. The surface expression of human 4-1BB in these cells compared to their parental cell line has been validated using flow cytometry **(Figure 1). Raji-Null cells** naturally express 4-1BBL, the ligand for 4-1BB, as validated by flow cytometry **(Figure 2)**. In the presence of an anti-h4-1BB antagonist antibody, the 4-1BB/4-1BBL-mediated activation is disrupted and there is no Lucia production by the Jurkat-Lucia™ h4-1BB effector cells **(Figure 3)**.

4-1BB expression on Jurkat-Lucia™ h4-1BB cells

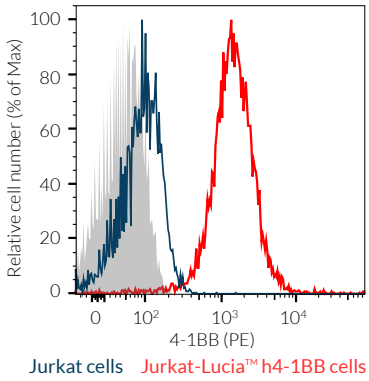

Figure 1: Validation of human 4-1BB surface expression by Jurkat-Lucia™ h4-1BB cells. Jurkat (blue) and Jurkat-Lucia™ h4-1BB (red) cells were incubated with a PE-conjugated Anti-h4-1BB mAb for 30 minutes. The binding affinity was measured using flow cytometry. Unstained cells are shown in grey.

4-1BBL expression on Raji-Null cells

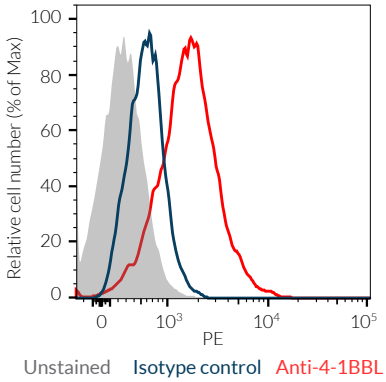

Figure 2: Validation of human 4-1BBL expression by Raji-Null cells. Raji Null cells were incubated with a PE-conjugated Anti-h4-1BBL mAb (red) or isotype control (blue) for 30 minutes. The binding affinity was measured using flow cytometry. Unstained Raji-Null cells are shown in grey.

Disruption of 4-1BB/4-1BBL activatory interaction using an anti-h4-1BB antagonist antibody

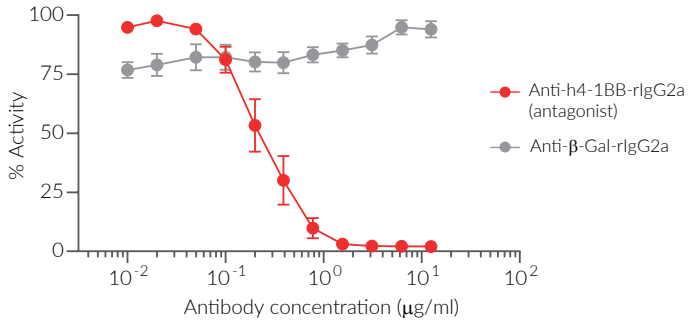

Figure 3: Activation of Jurkat-Lucia™ h4-1BB cells. Jurkat-Lucia™ h4-1BB cells cells were incubated with gradient concentrations of antagonist Anti-h4-1BB-rIgG2a or control Anti-β-Gal-rIgG2a monoclonal antibodies for 1 hour before the addition of Raji-Null cells. After 24 hours, the NF-κB activation in Jurkat-Lucia™ h4-1BB cells, reflecting the interaction of 4-1BBL on Raji-Null with the 4-1BB receptor on the Jurkat effector cells was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ 4. Percentages of the maximal responses are shown (mean ± SEM).

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 Asia: +852 3622-3480
E-mail: info@invivogen.com

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www.invivogen.com