# Validation data for Jurkat-Lucia™ NFAT-CD28 Cells

https://www.invivogen.com/jurkat-lucia-nfat-cd28-cells

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Version 23J25-AK

Jurkat-Lucia™ NFAT-CD28 cells were designed as reporter cells for CD3 (signal 1) and CD28 (signal 2) trigerred nuclear translocation of NFAT (nuclear factor of activated T-cells). They were engineered from a Jurkat clone deficient for CD28 expression. They were obtained by stable transfection of Jurkat-Lucia™ NFAT cells with the gene encoding human CD28. Jurkat-Lucia™ NFAT-CD28 cells also stably express the Lucia luciferase reporter gene under the control of an ISG54 minimal promoter fused to six NFAT response elements. Human CD3 and CD28 expression by Jurkat-Lucia™ NFAT-CD28 cells has been verified by flow cytometry **(Figure 1).** These cells have been functionally tested using antibody-mediated CD3 and CD28 cross-linking and measuring the levels of Lucia luciferase secreted in the supernatant **(Figure 2)**.

**CD3 and CD28 expression on Jurkat-Lucia™ NFAT-CD28 cells**

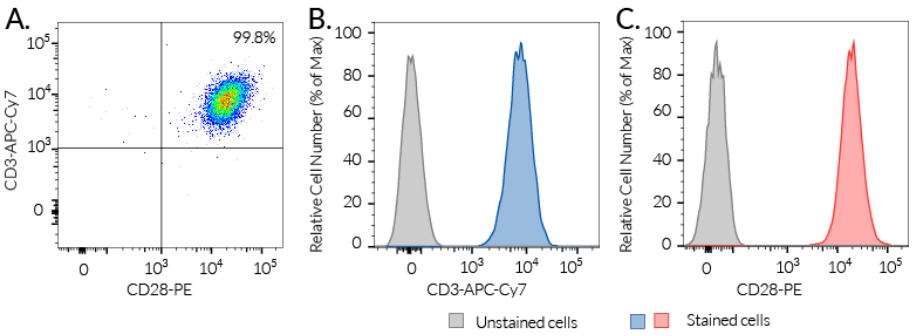


**Figure 1. Validation of human CD3 and CD28 cell surface expression by Jurkat-Lucia™ NFAT-CD28 cells.** Jurkat-Lucia™ NFAT-CD28 cells were incubated with a cocktail of PE-conjugated anti-hCD28 and APC-Cy7-conjugated anti-hCD3 antibodies for 30 minutes. The binding affinity was then measured using flow cytometry. **(A)** Co-expression of CD3 and CD28, **(B)** CD3 and **(C)** CD28 expression compared to unstained cells.

**Jurkat-Lucia™ NFAT-CD28 cell responses to antibody-mediated CD3 and CD28 cross-linking**

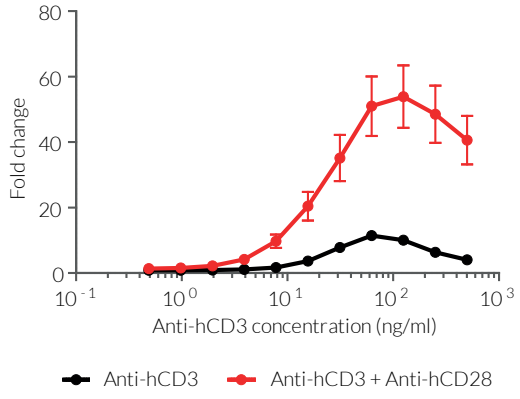


**Figure 2. Validation of NFAT activation in Jurkat-Lucia™ NFAT-CD28 cells.** Jurkat-Lucia™ NFAT-CD28 cells were incubated with increasing concentrations of anti-hCD3 mAb only (black) or with 0.5 µg/ml anti-hCD28 mAb (red) for 6 hours. NFAT activation was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Fold change over non-induced cells is shown (mean±SEM).


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