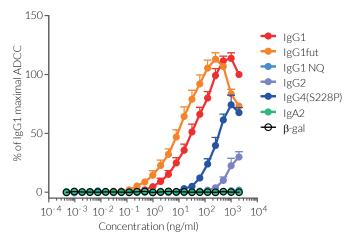
## Validation data for Jurkat-Lucia™ NFAT-CD16 Cells

https://www.invivogen.com/jurkat-lucia-nfat-cd16-cells
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Version 19804-NJ

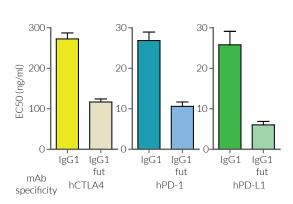
Jurkat-Lucia™ NFAT-CD16 cells were engineered from the human T-lymphocyte Jurkat cell line and designed as reporter cells for antibody-dependent cellular cytotoxicity (ADCC). Nuclear translocation of NFAT (nuclear factor of activated T-cells), a transcription factor naturally expressed by Jurkat cells, is an early signaling event in ADCC induction. Jurkat-Lucia™ NFAT-CD16 cells stably express the cell surface Fc receptor CD16A (FcγRIIIA; V158 high affinity allotype) and the Lucia luciferase reporter gene under the control of an ISG54 minimal promoter fused to six NFAT response elements. Human CD16A expression by Jurkat-Lucia™ NFAT-CD16 cells has been verified by flow-cytometry. These cells have been functionally tested with various target cells and specific monoclonal antibody (mAb) isotype combinations. Antibodies displaying lower EC50 have higher ADCC potency..

## Jurkat-Lucia™ NFAT-CD16 cell responses to ADCC induction with anti-human CD20 isotypes and Raji-hCD20 target cells



Comparison of ADCC potency for native and engineered anti-human CD20 isotypes: Raji-Null cells were incubated with gradient concentrations of Anti-hCD20 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.

## EC<sub>50</sub> for different antibodies inducing ADCC using Jurkat-Lucia™ NFAT-CD16 reporter cells



Increased ADCC activity mediated by IgG1 compared to IgG1fut (non-fucosylated): Raji-hCTLA4, Raji-hPD-1, and Raji-hPD-L1 cells were incubated with Jurkat-Lucia $^{\text{TM}}$  NFAT-CD16 effector cells and corresponding IgG1 or IgG1fut specific mAbs. The data represent the EC $_{50}$  for each antibody.



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