Cell Line Description
Jurkat T cells inducibly expressing firefly luciferase under the control of NFAT response elements driven by the activation signals from **Cat (*Felis catus*) FcγRIIIA** and human Fcγ common chain. The genes have been transduced by lentivirus (TdTomato positive marker) and maintained by antibiotics G418 and Hygromycin B.

Receptor Gene
Cat FcγRIIIA. https://www.uniprot.org/uniprot/Q9N2I5

Culture Protocol
Cells should be cultured in RPMI 1640-10%FBS supplemented L-Glutamine (4 mM) and Pen/Strep (100 U/mL), plus G418 (100 µg/mL) and Hygromycin B (200 µg/mL). Viable cells tend to form aggregates.

Applications
Characterize the Fc effector function of antibodies and measure ADCC activity in cellular assays.

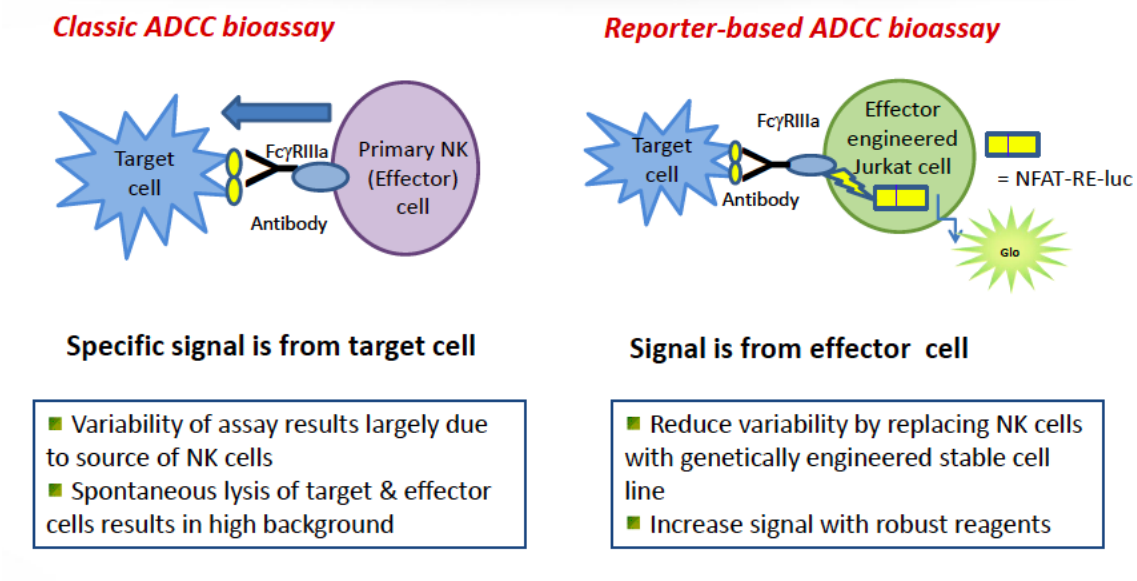
ADCC Assay Protocol
One day before the assay, target cells expressing the cognate antigen are seeded at 10,000 cells/well into Falcon white opaque 96-well plate (Cat. # 353296) in 100 µL medium and cultured at 37°C for overnight. On the next day, 75 µL medium is removed from each well, and 200,000 Jurkat reporter cells/25 µL/well plus 25 µL of antibodies with 4-fold serial dilutions (from 10.0 or 1.0 µg/mL highest concentration) are added, and the cell mixtures are cultured at 37°C for 6 hrs. At the end of the culture, the plates are cooled at room temperature for 15 min, and 60 µL Bio-Glo luciferase assay buffer (Promega, Cat. # G719A) is added to each well. Luciferase signals are read on a luminometer within 5-30 min.

Storage/Stability
Culture expanded cells can be frozen in 90%FBS+10%DMSO at -150°C. As long as properly cultured in G418 and Hygromycin B supplemented RPMI medium, cell phenotype (TdTomato+) and functionality can be maintained for long term.

Background
Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune defense mechanism involving an effector cell lysing a target cell on which antibodies have bound to specific antigens on the target cell membrane.

The classical ADCC assay measures the lysis of target cells, using release of labeled radioactivities (^{51}Cr) or enzymes (such as LDH) as readout. Nowadays, measuring the activation of effector cells upon Fc receptor engagement and crosslinking by luciferase signals is more routinely adopted to replace the classical method.

Assay Principle:



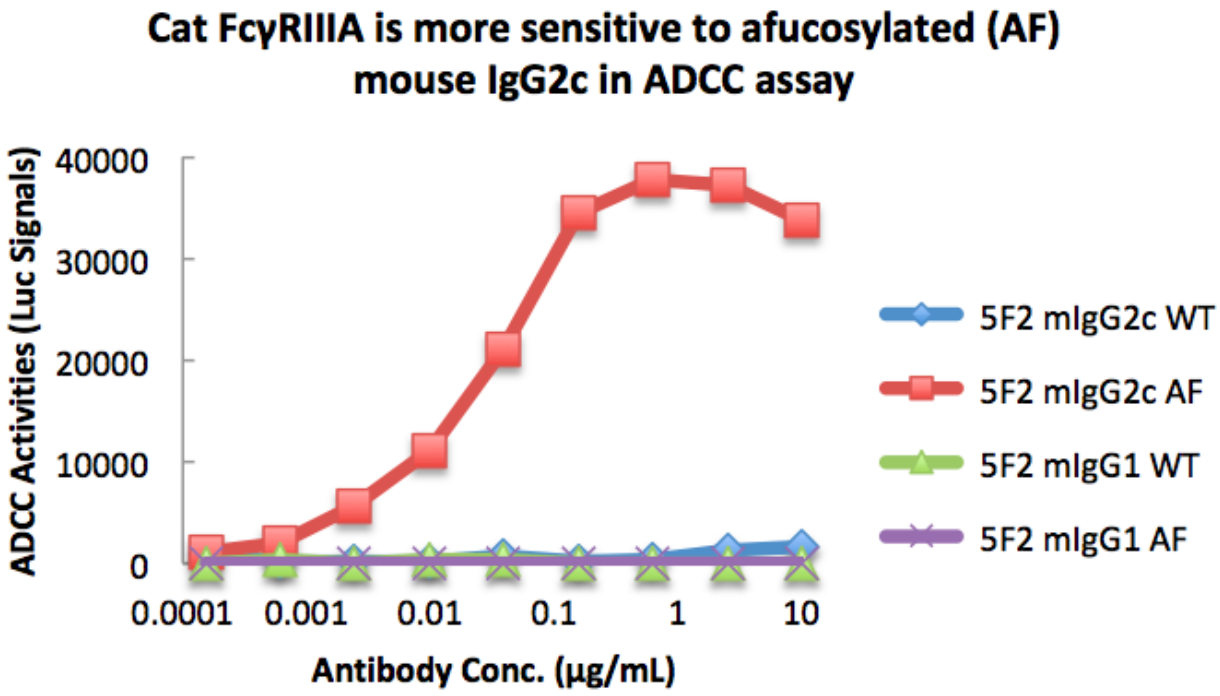
Specific signal is from target cell

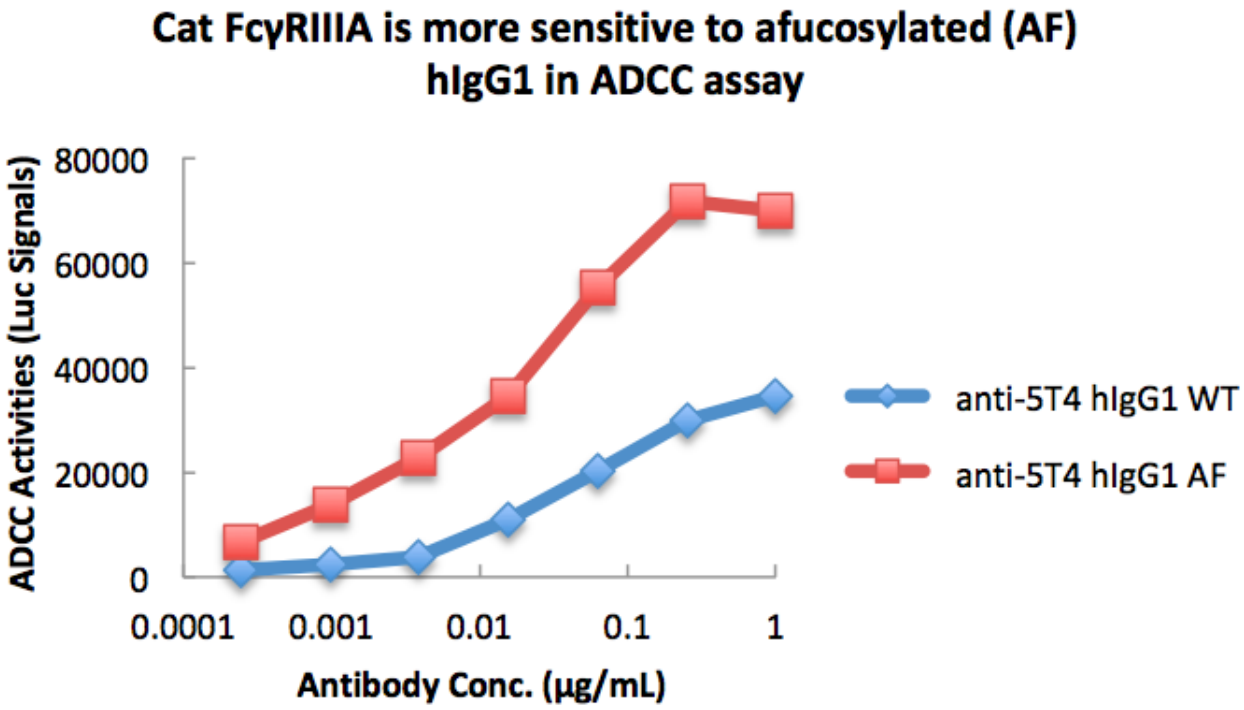
- Variability of assay results largely due to source of NK cells
- Spontaneous lysis of target & effector cells results in high background

Signal is from effector cell

- Reduce variability by replacing NK cells with genetically engineered stable cell line
- Increase signal with robust reagents

Assay example





Reference

Cross-species higher sensitivities of FcγRIIIA/FcγRIV to afucosylated IgG for enhanced ADCC. Mao C, Near R, Zhong X, Gao W. *Antib Ther.* 2021 Aug 19;4(3):159-170. doi: 10.1093/abt/tbab016. https://academic.oup.com/abt/article/4/3/159/6354750?login=true

Notes

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Warnings
Avoid freeze/thaw cycles.