Advanced Assay Platform for Antibody Development:
Precision Assays for ADCC, ADCP, and CDC

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SVAR
Answers in Life Science

INTRODUCTION

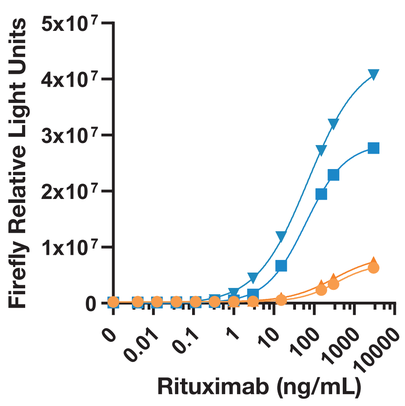
Early in therapeutic antibody development, it's crucial to characterize their mode of action (MoA). The Fc regions of antibodies significantly influence their therapeutic characteristics, including half-life and cytotoxic effects like ADCC, ADCP, and CDC.

To streamline this evaluation, we've developed advanced assays for ADCC, ADCP, and CDC activities. Our proprietary Fc Effector assay platform, leveraging *iLite*® technology, uses gene-reporter effector cells with custom-engineered antigen-positive and antigen-negative target cells. This ensures superior specificity, accuracy, precision, and linearity, ideal for immuno-oncology drug development. Comparative studies show our *iLite* ADCC and ADCP assays outperform competitors in sensitivity, dynamic range, and ease of use, effectively capturing the MoA of therapeutics like rituximab, daratumumab, and alemtuzumab.

Additionally, our CDC assay uses a cell line engineered from a complement-competent line to express SVAR luciferase (SL). This cell line enables the creation of fast, robust, and reproducible functional assays for CDC, and can be customized to express any cell surface antigen.

Traditional ADCC and ADCP bioassays rely on human primary cells, which suffer from donor variability, limited expansion capacity, and labor-intensive culturing. Our trifecta of advanced assays—ADCC, ADCP, and CDC—provides researchers with reliable, consistent, and customizable tools for comprehensive evaluation of therapeutic antibodies, significantly accelerating drug development in immuno-oncology.

PERFORMANCE OF THE CELL LINES

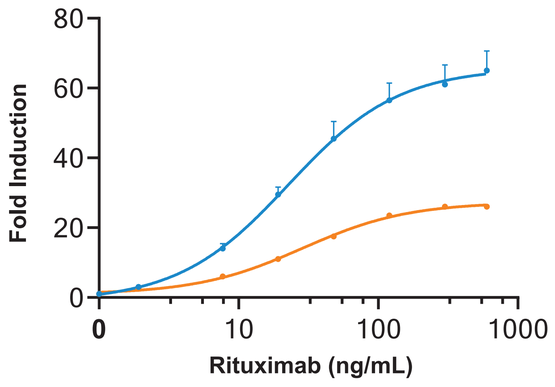


ILITE ADCC VS COMPETITOR CD20

We compared our novel *iLite*® ADCC assay to that of a leading competitor using the same target cells and therapeutic antibody.

As seen in the graph, ***iLite* ADCC assay provides a significantly increased dynamic range and sensitivity** compared to the bioassay from a leading competitor.

- Competitor ADCC Effector cells + Raji
- *iLite* ADCC Effector cells + Raji
- Competitor ADCC Effector cells + CD20(+)
- *iLite* ADCC Effector cells + CD20(+)

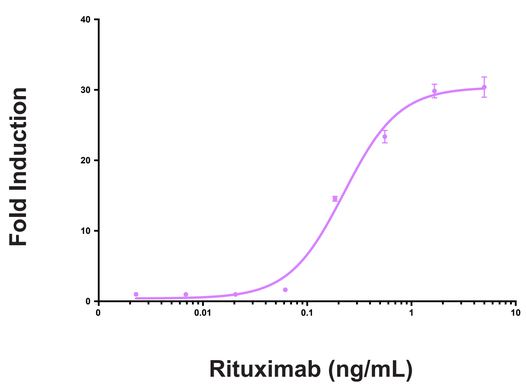


ILITE ADCP VS COMPETITOR CD20

We compared our novel *iLite*® ADCP assay to that of a leading competitor using the same target cells and therapeutic antibody as before.

As seen in the graph, ***iLite* ADCP effector cells offer higher fold induction and a similar EC50 value** when compared to effector cells from a leading competitor.

- *iLite* effector + CD20(+) target cells
- Competitor effector + CD20(+) target cells



ILITE CDC INDUCTION BY RITUXIMAB

The CD20+SL target cell line is endogenously expressing several relevant surface antigens recognized by drug antibodies capable of inducing complementdependent cytotoxicity (CDC) such as CD20, CD38 and to a lesser extent CD52.

As seen in the graph, ***iLite*® CDC cell line can be used to detect CDC-mediated killing.**

PRINCIPLE OF THE FC EFFECTOR CELLS

The ADCC/ADCP process is triggered when the effector cell interacts with a drug antibody bound to its target cell. An FcR (CD16 on ADCC effector cells and CD32 on ADCP effector cells) on the surface of the effector cell binds to the Fc region of the antibody, thus creating a bridge between the effector and target cells.

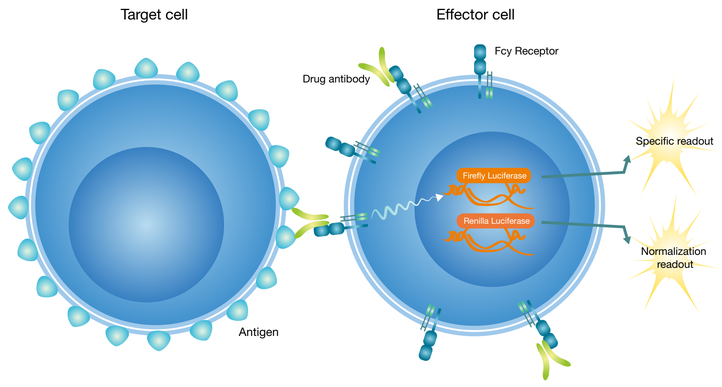


Figure 1. *Schematic representation of an iLite ADCC/ADCP bioassay.*

Following the formation of this bridge, the engineered effector cells will, instead of lysing the target cells as in the in vivo situation, produce luciferase through an intracellular pathway and generate luminescence exclusively from this cross-linking and signaling. The strength of the luminescence correlates to the ability of the drug to induce ADCC.

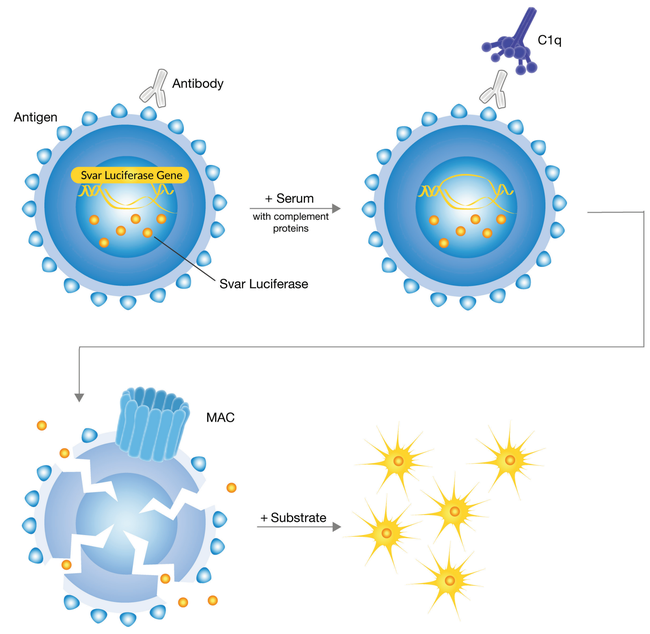
PRINCIPLE OF THE CDC BIOASSAY

Our CDC cell lines express Svar luciferase from a constitutive promoter. In addition, they overexpress antigens on the cell surface that can be bound by a therapeutic drug antibody.

Complement proteins from the added human serum can bind to the antibody, triggering lysis of the cell in a process like that of the body.

Upon lysis, luciferase leaks out of the cell and can be measured following the addition of a substrate.

Figure 2. *Schematic representation of a CDC assay.*

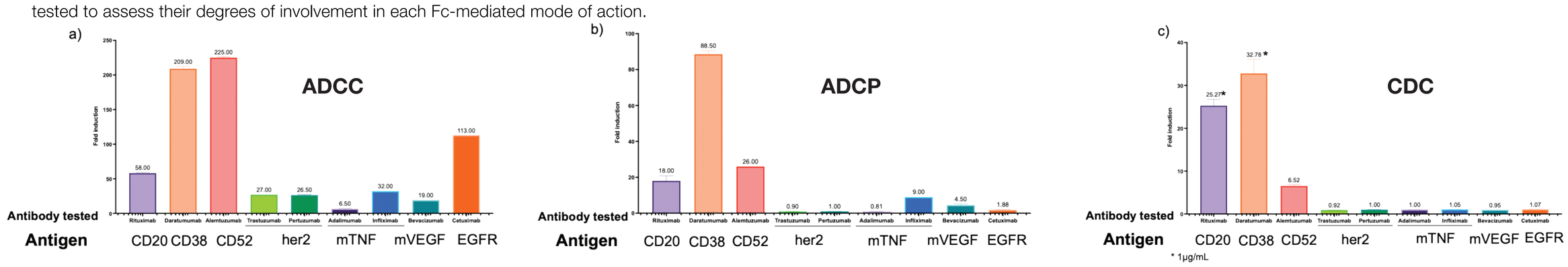


ADCC, ADCP & CDC ASSAY PERFORMED WITH THE SAME TARGET CELLS

ADCC, ADCP or CDC assays were performed using *iLite*® ADCC and ADCP effector cell lines or human serum with the CD20+SL target cell line, respectively.

The *iLite* effector cell lines are reporter cell lines expressing Firefly Luciferase as a reaction on antibody mediated and target-cell dependent cell signalling. Corresponding therapeutic antibodies have been tested to assess their degrees of involvement in each Fc-mediated mode of action.

Antibody-mediated ADCC and ADCP are measured by the activity of firefly Luciferase expressed by the iLite ADCC and ADCP effector cells, respectively. Antibody mediated CDC (c) is measured by the release of Svar luciferase of the lysed cells. Of note, also in the context of ADCC and ADCP assays our CD20+SL target cells can be used in real killing assays. Data not shown here.



CONCLUSION

We have developed a robust and versatile platform for functional assessment to detect Fc-mediated ADCC, ADCP, and CDC through luciferase detection. The first setup—a dual cell system with effector and target cells—is suitable for determining ADCC and ADCP activities in various assay designs.

Additionally, luciferase-containing target cells are used to assess CDC activity. The platform allows rapid customization by adding surface antigens of interest to the target cells, enabling the evaluation of Fc-mediated cytotoxic mechanisms of any antibody.

Our novel suite of cells fully reflects the MoA of therapeutics like rituximab while offering superior sensitivity and dynamic range compared to leading competitors. The *iLite* effector and target cell systems are well-suited for applications such as potency assays in CMC environments and for quantifying ADCP and ADCC activities in pre-clinical or clinical studies. The inclusion of a luciferase normalization gene aids in compensating for serum matrix effects, ensuring reliable results even in challenging clinical samples.

Overall, our advanced assay platforms for ADCC, ADCP, and CDC provide researchers with consistent, customizable tools for comprehensive antibody evaluation, significantly accelerating the development of therapeutic antibodies in immuno-oncology and other medical fields.

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