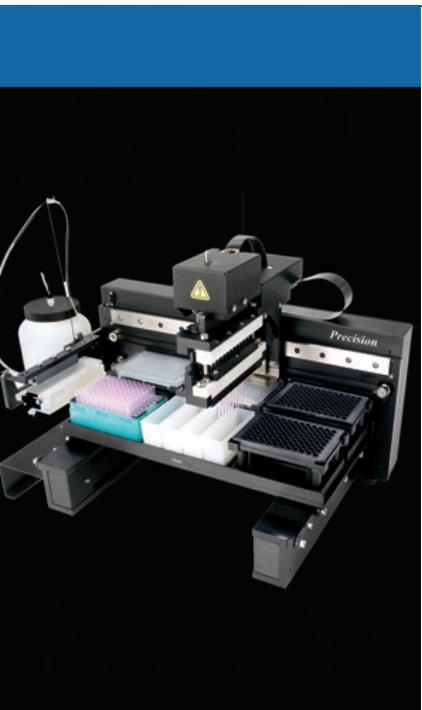




# Automated Bioluminescent ADCC Reporter Bioassay Using Bioengineered Jurkat Cells

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## Introduction

Pharmaceutical companies are increasingly exploring new biologic and biosimilar products, and thus increasing monoclonal antibody (mAb) immunotherapeutic research. In keeping with this trend, the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA) have each drafted guidelines<sup>1,2</sup> for biosimilars product development. Both recommend extensive structural and functional characterization of the proposed therapy, demonstrated in part through antibody-dependent cell-mediated cytotoxicity (ADCC) assays, complement-dependent cytotoxicity (CDC) assays and complement activation to "cover all functional aspects of the mAb." The body uses these cytotoxicity mechanisms of action (MOA) to destroy cells that could otherwise proliferate and cause disease or other maladies.

Here we discuss the principles of a bioluminescent ADCC bioassay used in mAb immunotherapeutic development, and demonstrate how this automated, non-radioactive, cell-based assay provides simple, safe, and reliable methods and results using bioengineered effector cells. The cells are engineered to express the FcγRIIIa receptor, the main Fc receptor (FcR) indicated in ADCC mechanism of action, thereby providing similar results as primary NK cells, without the increased time and effort associated with primary cell purification steps. Additionally, when the assay is automated using BioTek's Precision™ Microplate Pipetting System and Synergy™ Multi-Mode Microplate Readers, the process is streamlined with less active labor required, and results are more robust than manual methods.

## ADCC Reporter Bioassay Principle

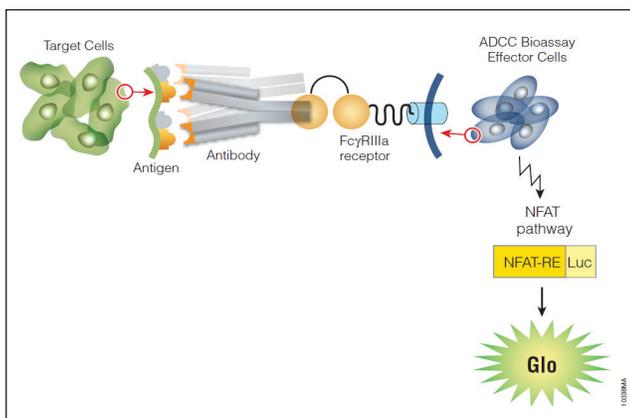
The ADCC Reporter Bioassay from Promega Corporation (Madison, WI) uses engineered, immortalized T lymphocyte Jurkat cells, expressing the FcγRIIIa receptor, instead of traditional NK cells. Specific antibodies bind the target cell at the Fab ends. The Fc region of the same antibodies then binds the FcγRIIIa receptor on the engineered effector cell. This receptor binding activates gene transcription through the nuclear factor of activated T-cells (NFAT) pathway in the effector cell leading to luciferase production.

In this assay, the effector cells are lysed by addition of the Bio-Glo™ detection reagents and the luciferase interacts with luciferin and ATP in solution to generate a stable luminescent signal.

The assay is a mix-and-read format, does not require centrifugation or transfer steps, and is representative of ADCC MOA. Additionally, the assay eliminates the need for radioisotope-labeled cells, such as those seen in classic Chromium-51-based assays.

### Key Words:

- ADCC
- Antibody-dependent Cell-mediated Cellular Cytotoxicity
- Antibody Therapeutic
- CD16
- FcγRIIIa
- Luminescence
- Automation



**Figure 1.** ADCC Reporter Bioassay principle showing antibody binding to target cell antigens and FcγRIIIa receptors expressed on engineered Jurkat effector cells. Luminescent signal is generated following specific antibody binding to target and effector cells.

## Materials and Methods

### Materials

#### Cells and Reagents

Complete assay kits comprised of thaw-and-use target cells (Raji or WIL2-S), thaw-and-use effector cells (engineered Jurkat), control antibody, detection reagent, and assay buffer components, were provided by Promega Corporation. The ADCC Reporter Bioassay, Complete Kit (Raji) - Catalog No. G7015, and ADCC Reporter Bioassay, Complete Kit (WIL2-S) – Catalog No. G7014, were used in this study.

#### Labware

White 96 Well, TC-Treated Microplates (Catalog No. 3917) from Corning Life Sciences (Corning, NY) were used to perform the assay, while 96 Well, Clear, Untreated Microplates from Corning (Catalog No. 3370) were used for performing the control antibody titration. A 12-column reservoir from Corning/Axygen (Catalog No. Res-MW12-HP-SI) was used to hold Assay Buffer, cell suspensions, and detection reagent.

#### Instrumentation

##### Precision™ Microplate Pipetting System

BioTek's compact Precision™ Microplate Pipetting System automates pipetting processes for walk-away operation. A user-configurable deck and four liquid handling transfer tools provide flexible experimental design for the ADCC reporter bioassay and many other microplate-based assays. Its small footprint allows for easy insertion into laminar flow hoods, making it especially suited for use with cell-based assay procedures. Per the ADCC reporter bioassay, Precision was used to perform all liquid handling steps, including test antibody titration and transfer, as well as target and effector cell and reagent dispensing.

##### Synergy™ Multi-Mode Microplate Readers

A variety of BioTek's Synergy™ Multi-Mode Microplate Readers are suitable for many budgets and applications. Each incorporates high precision and performance in fluorescence, luminescence and absorbance modes, and are driven by integrated Gen5™ Data Analysis Software. The Synergy H1 and Synergy Mx Multi-Mode Microplate Readers are especially useful for Promega's ADCC reporter bioassay. Both provide high sensitivity and flexibility for cell-based and biochemical assays, with UV-visible absorbance, luminescence and quadruple grating monochromator optics for top and bottom fluorescence. Synergy H1 may be upgraded at any time with an optional filter-based optics module for advanced read modes like fluorescence polarization, time-resolved fluorescence and time-resolved fluorescence resonance energy transfer.

The optics are devoid of fiber optic cables, reflective mirrors and other components that limit light transmission and reduce sensitivity.

### Methods

#### Cell Preparation

Frozen Raji and WIL2-S target cells, and frozen Jurkat effector cells, were thawed carefully for 2 minutes in a 37 °C water bath. A suspension of WIL2-S cells was prepared by adding 0.5 mL of cells to a 15 mL conical tube containing 7.5 mL of Assay Buffer. A suspension of Raji cells was prepared by adding 0.5 mL of cells to a 15 mL conical tube containing 9.5 mL of Assay Buffer. Jurkat effector cells were prepared by adding 0.63 mL of cells to 3.6 mL of Assay Buffer.

#### Antibody Preparation

The Anti-CD20 Control Antibody was prepared by adding 50 µL of tissue culture grade water to the vial to make a stock concentration of 100 µg/mL. Two different working dilutions of the antibody were prepared for each assay. For the WIL2-S assay, the antibody was diluted in ADCC Assay Buffer to a 3X working concentration of 6 µg/mL. For the Raji assay, the antibody was diluted in ADCC Assay Buffer to a 3X working concentration of 9 µg/mL.

#### Automated Antibody Dilution Procedure

3X antibody was manually added to the antibody titration plate. The plate was transferred to the Precision for diluent addition and antibody titration. Assay Buffer diluent was added to the empty columns of the titration plate. For the WIL2-S assay, antibody was titrated 1:3 across the plate. For the Raji assay, antibody was titrated 1:2.5 across the plate. A 10-point titration curve, including a no-antibody control, was created for each assay.

#### Automated Assay Procedure

Twenty-five microliters of target Raji or WIL2-S cells were added to the wells of a 96-well assay plate followed by transfer of 25 µL of titrated anti-CD20 monoclonal antibody. Twenty-five microliters of Jurkat effector cells were then added to each well, and the plates were incubated for 6 hours at 37 °C, 5% CO<sub>2</sub>. Both Raji and WIL2-S assays were performed with an effector to target cell ratio (E:T) of 6:1. After incubation, the plate was cooled to room temperature for 15 minutes, and 75 µL of Bio-Glo™ Reagent was added. The plate was incubated for 5 minutes at room temperature, then read using Synergy Mx's luminescence mode. Data generated by Synergy Mx demonstrates similar data to that generated by Synergy H1. An increase in luminescent signal is therefore proportional to the binding level of the test antibody to the expressed FcγRIIIa receptor.

## Results

Manual and automated ADCC reporter bioassay methods were compared using Raji cells. As shown in Figure 2, manual- and automated-derived methods generated equivalent data, and  $EC_{50}$  results also closely correlate to previously generated data of  $0.743e-07$  g/mL<sup>3</sup>, thus validating the automated assay method. It is important to note that since antibody binding to the engineered effector cell is measured, data will not show percent total cytotoxicity. However, positive fold induction is seen with increased binding, and  $EC_{50}$  values are equivalent to those generated using traditional, manual methods.

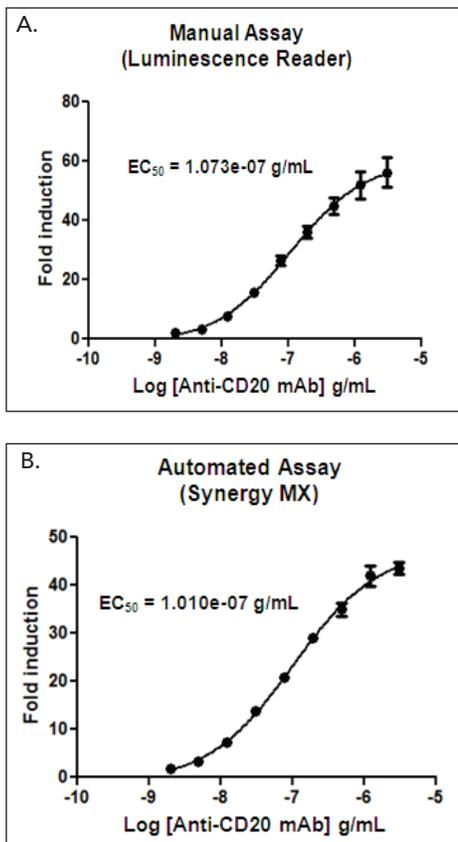


Figure 2. ADCC Reporter-based bioassay comparison of automated versus manual methods using Raji target cells.

Synergy Mx Multi-Mode Microplate Reader's performance was also compared to that of a luminescence-focused microplate reader (Figure 3). Equivalent fold induction and  $EC_{50}$  values ( $1.01e-07$  g/mL and  $8.787e-08$  g/mL, respectively) are seen across the readers, thus demonstrating Synergy Mx's high sensitivity and accuracy, along with its increased functionality.

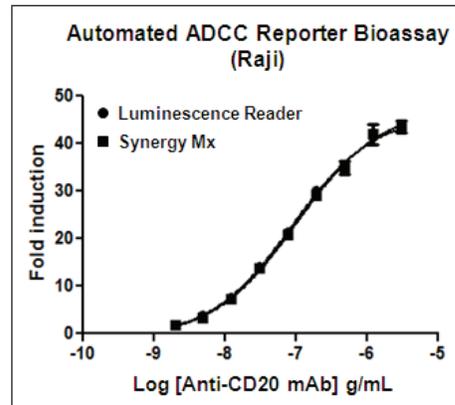


Figure 3. Comparison of results between a luminescence reader and Synergy Mx using Raji target cells.

Finally, the automated ADCC Reporter Bioassay was also validated using WIL2-S cells. As seen with the Raji cell validation data, WIL2-S cell  $EC_{50}$  values (Figure 4) are similar to the previously generated and manually-derived  $EC_{50}$  value of  $2.29e-08$ <sup>3</sup>. This demonstrates the accuracy and robustness of an automated method using the ADCC Reporter Bioassay technology.

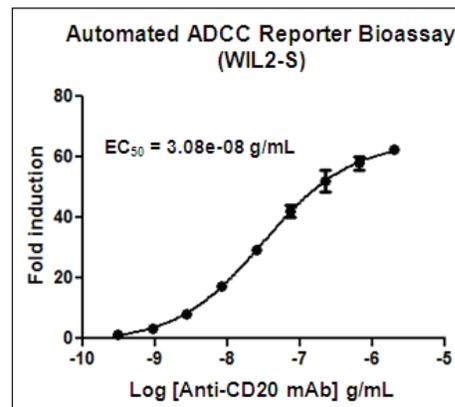


Figure 4. Automated ADCC Reporter Bioassay validation using WIL2-S target cells.

## Conclusions

We have demonstrated a simple, homogeneous, non-radioactive, mix-and-read assay platform for ADCC studies using luminescent detection and genetically engineered cells. The assay workflow can be easily automated with instruments like BioTek's Precision to increase throughput and repeatability. The various read modes in Synergy Multi-Mode Microplate Readers add even more flexibility to the processes, and increase efficiency to the overall laboratory workflow. The combination of assay chemistry, appropriate cell models, and automated instrumentation can increase productivity and throughput, simplify processes, and generate high-quality, reproducible results for the discovery of new antibody therapeutics.

## References

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