Performing ADCC Reporter Bioassay with an Environmentally Controlled Microplate Reader

Kevin Schiro[1], Tracy Worzella[1], Zhijie Jey Cheng[1], Denise Garvin[1], Teresa Surowy[1], Thomas Hengstl[2], Michael Fejtl[2], and Christian Oberdanner[2]

[1]Promega Corporation; [2]Tecan Austria GmbH

The Tecan Infinite® 200 PRO with Gas Control Module (GCM™).

Materials

- Promega ADCC Reporter Bioassay, Complete Kit (Raji), Cat.# G7015 or ADCC Reporter Bioassay, Complete Kit (WIL2-S), Cat.# G7014. Each kit contains:
  - •ADCC Bioassay Effector Cells
  - •ADCC Bioassay Target Cells (Raji or WIL2-S)
  - •Control Ab, Anti-CD20
  - •Low IgG Serum
  - •RPMI 1640 Medium
  - •Bio-Glo™ Luciferase Assay Buffer
  - •Bio-Glo™ Luciferase Assay Substrate
- White, 96-well, flat-bottom tissue culture plates with lid (Corning Costar Cat.# 3917)
- TECAN Inifinite® 200 PRO with GCM

Protocols:

ADCC Reporter Bioassay, Complete Kit, (Raji) Technical Manual #TM387 and *ADCC Reporter Bioassay, Complete Kit, (WIL2-S) Technical Manual* #TB86 Available at:
www.promega.com/protocols/

For more informaiton on the ADDC Reporter Bioassay Systems, please visit:
www.promega.com/adcc

Note: The ADCC Reporter Bioassay, Core Kit or Complete Kit, contains sufficient reagents for 120 assays using the inner 60 wells of two 96-well plates. The critical components in these kits are the single vials of effector and target cells. They are designed for one-time use and cannot be refrozen once thawed (i.e., single thaw and plating). The Infinite 200 PRO only accomodates a single 96-well plate during the six-hour induction. Please plan ahead before starting your assay.

Abstract

The ADCC Reporter Bioassay (Promega) provides effector and target cells in frozen, thaw-and-use format ("Complete kits"), removing the need for isolating or culturing cells in order to perform the assay. Reporter induction requires a six-hour to overnight incubation in a standard cell culture incubator. The carbon dioxide and temperature control features of the Infinite® 200 PRO with Gas Control Module (GCM, Tecan) allow the assay induction to be performed directly within the detection instrument, eliminating the requirement of a cell culture incubator.

Introduction

Antibody-dependent cell-mediated cytotoxicity (ADCC) is a mechanism of action (MOA) of antibodies through which tumor, virus-infected or other diseased cells are targeted for destruction by components of the cell-mediated immune system. Therefore, ADCC is a desirable mechanism for killing target cells using antibody-based drugs. Bioassays designed to measure ADCC are particularly useful for biopharmaceutical companies and contract research and manufacturing organizations looking to understand and characterize mechanism of action, and to quantify biologic potency and stability of target compounds during discovery and development.

Commercially available assay reagents are facilitating bioassay ease-of-use, but the labor and skill required to isolate primary natural killer cells for ADCC or to maintain alternative cell lines in culture with any level of reproducibility remains problematic. Long-term cell maintenance carries costs in consumable reagents (e.g., media, plasticware), but also costs in purchasing and maintaining basic equipment such as biosafety cabinets, centrifuges and tissue culture incubators.

Promega has developed reporter bioassays that use activation of gene transcription through NFAT (nuclear factor of activated T-cells) as an earlier measure of ADCC. The reporter bioassays provide a convenient system for assessing ADCC that uses frozen, thaw-and-use effector and target cells and eliminates the need for cell culture maintenance. The use of frozen cells also decreases assay variability, which results from cells grown in continuous culture or from the use of primary cells from individual donors. The ADCC Reporter Bioassay described here uses engineered Jurkat effector cells stably

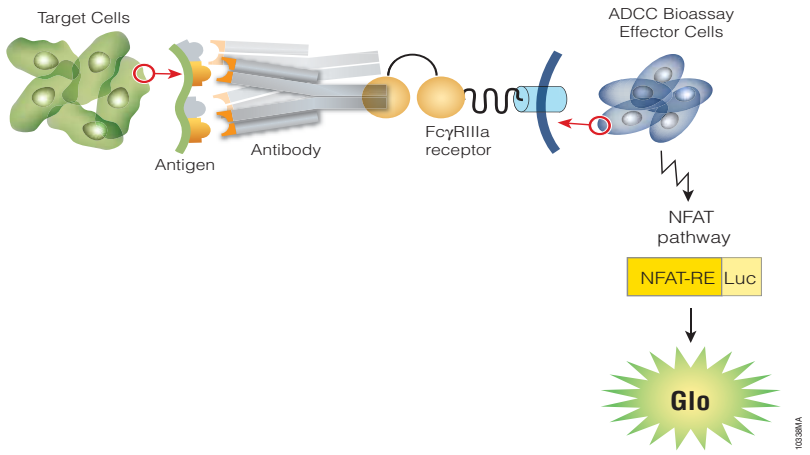

Figure 1. Principle of the ADCC Reporter Bioassay.

expressing the V158 variant of the FcγRIIIa receptor, and an NFAT response element driving expression of firefly luciferase. Antibody biological activity in ADCC MOA is detected with the addition of Bio-Glo™ reagent, which is converted to a luminescent product in the prescence of NFAT pathway activiation and quantified as luminescence readout (Figure 1). Both Raji and WIL2-S target cells are available in complete kit assays.

The ADCC Reporter Bioassay requires a six-hour incubation with cells and antibody for optimal NFAT activation and luciferase expression to occur. The induction is typically performed within a standard cell culture incubator where temperature, humidity and carbon dioxide (CO_2) are controlled. Here we evaluate an alternative method of performing the pre-detection, in-plate cell response induction using detection instrumentation equipped with temperature and gas control. This alternative method enables scientists without access to a cell culture facility to easily perform the bioluminescent reporter bioassay.

The Infinite® 200 PRO with GCM is a multimode reader equipped with an external module that enables simultaneous adjustment of CO_2 and O_2. The module monitors partial pressure of these gases by injecting CO_2 and N_2 into the detection chamber from attached CO_2 and N_2 tanks. The high sensitivity and precision offered by the system automatically compensates for variations in the atmospheric partial pressure of CO_2 using the module's unique altitude correction function, which is accessed by an intuitive user interface. Rapid detection of changes in gas pressure or flow will indicate if the target concentration will not be reached or deviates significantly during incubation, at which point both audible and visible warnings alert the user. The onboard temperature control allows precise temperature adjustment up to 42°C. This multimode reader is equipped with Quad4 Monochromators™ for finely tuned wavelength selection. Linear and orbital shaking help ensure well contents are homogeneous before each read.

Methods

Plate Setup: Only the inner 60 wells of each assay plate were used for the experiments; assay buffer (RPMI 1640 Medium + 4% Low IgG Serum) was added to the perimeter wells of two 96-well microplates. All additions to the assay plates were performed manually. Since the Tecan reader lacks humidity control, we added 150µl of assay buffer to the inter-well spaces of one plate, which was later placed in the Tecan reader. Target cells were thawed in a 37°C water bath for two minutes then added to assay buffer. The cell suspensions were then added to both assay plates using the same plate layout, Raji cells in rows B–D and WIL2-S cells in rows E–G. A dilution series of Control Antibody, Anti-CD20, was added to both plates in final 1X concentrations ranging from 6µg/ml–0.73ng/ml for Raji cells and 4µg/ml–0.14ng/ml for WIL2-S. Wells without antibody served as a negative control, while wells containing buffer only served as reagent background control. Effector cells were prepared in the same manner as the target cells and added to the inner 60 wells of both plates. Plates were briefly mixed on an orbital shaker. The plate containing media in the inter-well spaces was placed in the Infinite® 200 PRO with GCM and incubated at 37°C/5% CO_2 for six hours. The second plate was placed in a tissue culture incubator at 37°C/5% CO_2 for six hours. During the six-hour incubation, Bio-Glo™ Luciferase Assay Buffer was thawed and added to Bio-Glo™ Luciferase Assay Substrate.

Plate Read: After the incubation period, plate handling was staggered so that each plate was processed in succession.

Each plate was equilibrated to ambient temperature for 15 minutes. Bio-Glo™ reagent was added to the inner 60 and background control wells. The internal temperature of the reader was held at 37°C. The lid was removed and each plate placed into the Tecan Infinite® 200 PRO for five minutes before reading luminescence. The normal recommendation for handling bioluminescent assay plates is to equilibrate both the assay plate and reader to ambient temperature prior to reading. Due to the extended time needed to equilibrate the Tecan reader from 37°C to ambient temperature (>2 hours), we elected to keep the reader at the elevated temperature during the short incubation after reagent addition.

Table 1. Instrument Settings for Incubation

Parameter	Set Point
Temperature	37°C
Gas control: CO_2/O_2	5%/ambient
Incubation	Six hours

Table 2. Instrument Settings for Detection

Parameter	Set Point
Temperature	37°C
Gas control: CO_2/O_2	5%/ambient
Read mode	Luminescence, lid removed
Integration time	500ms
Equilibration time before read	Five minutes
Read duration	Once, after six-hour incubation

Results

We determined the fold induction of response over the untreated control for each concentration of antibody tested. Data were fitted with GraphPad Prism® software. The ADCC Reporter Bioassays with Raji or WIL2-S target cells incubated in the Infinite® 200 PRO Reader performed comparably, in both fold induction and potency, to the control plate incubated in a standard tissue culture incubator (Figure 2). The potencies obtained with Raji cells were 66ng/ml (200 PRO/GCM) and 78ng/ml (standard tissue culture), well within the typical range for the assay of 10–158ng/ml. Potencies with the WIL2-S cells were 36ng/ml (200 PRO/GCM) and 37ng/ml (standard tissue culture), also within the typical range for that assay at 6–68ng/ml.

Conclusion

We found the Infinite® 200 PRO Reader with GCM to be functionally equivalent to a tissue culture incubator for the reporter induction phase of the ADCC Reporter Bioassay. Fold induction and potency of response were comparable between the two incubation methods for both Raji and WIL2-S target cells used in the bioassay. Our study showed that the convenience of using a kit containing frozen, thaw-and-use cells was further enhanced by incorporating instrumentation that enabled both temperature and gas-control to mimic conditions provided by a standard cell culture incubator. Although humidity control was not provided by the reader, we were able to compensate by adding assay buffer to the inter-well spaces of the assay plate. Using the complete kit with an environmentally controlled reader demonstrates the ability to perform the ADCC Reporter Bioassay in laboratories lacking standard cell culture incubators.

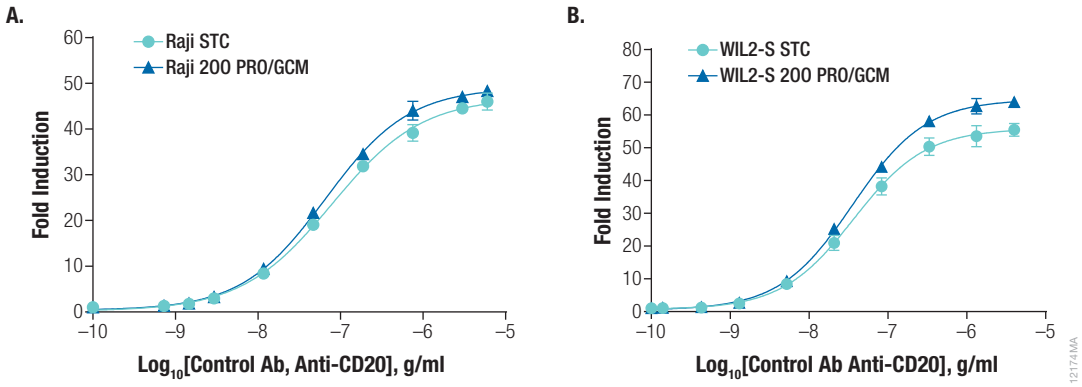

Figure 2. ADCC Reporter Bioassay, conducted using the Infinite® 200 PRO Reader with GCM (200 PRO/GCM) performed equivalently to assays incubated in a standard tissue culture (STC) incubator.