Measuring the ADCC Reporter Bioassay, Complete Kit (WIL2-S) Signal on the GloMax® Discover System

Promega Corporation

Materials Required

- ADCC Reporter Bioassay, Complete Kit (WIL2-S) (Cat.# G7014)
- GloMax® Discover System (Cat.# GM3000)
- Sterile, clear 96-well, V-bottom plate with lid (Linbro Cat.# 76-223-05 or equivalent) for preparing antibody dilutions
- White, flat-bottom, 96-well assay plates (Corning Cat.# 3917 or equivalent)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *GloMax® Discover System Technical Manual* #TM397 is available at: **www.promega.com/protocols/**

Antibody-dependent cell-mediated cytotoxicity (ADCC) is a mechanism of action of antibodies through which virus-infected or other diseased cells are targeted for destruction by components of the cell-mediated immune system, such as natural killer cells. The GloMax® Discover System used in conjunction with the ADCC Reporter Bioassay provides an optimized bioluminescent procedure for quantifying biological activity on pathway activation by therapeutic antibody drugs in an ADCC mechanism of action (MOA) assay.

The ADCC Reporter Bioassay uses an alternative readout at an earlier point in ADCC MOA pathway activation than classic ADCC bioassays, which use donor peripheral blood mononuclear cells (PBMCs) or the natural killer (NK) cell subpopulation as effector cells. The ADCC Reporter Bioassay, instead, utilizes engineered Jurkat cells stably expressing the FcγRIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase as effector cells. Antibody biological activity in ADCC MOA is quantified through the luciferase produced as a result of NFAT pathway activation; luciferase activity in the effector cell is quantified with luminescence readout. Signal is high, and assay background is low.

The ADCC Reporter Bioassay is made easy on the GloMax® Discover System, and the protocol comes preloaded on the instrument. The extended dynamic range of the GloMax® Discover System enables sensitive detection over a range in luminescent signal intensities using the ADCC Reporter Bioassay. This Application Note describes the protocol for measuring luminescence using the GloMax® Discover System with the ADCC Reporter Bioassay Complete Kit (WIL2-S).

Experimental Protocols

Preparation of Components, Reagents and Bioassay Starting Materials

- **Bio-Glo™ Luciferase Assay Reagent:** Equilibrate Bio-Glo™ Luciferase Assay Buffer and Bio-Glo™ Luciferase Assay Substrate to ambient temperature, combine, and mix by inversion.
- **ADCC Assay Buffer:** Thaw the Low lgG Serum in a 37°C water bath and add 1.4ml to 33.6ml of RPMI 1640 Medium. Mix well and warm to 37°C prior to use.

- **Reconstitution and starting dilution of Control Ab, Anti-CD20:** Transfer 50µl of tissue-culture-grade water to the vial to bring the antibody stock solution to 100µg/ml. Gently swirl to mix, then place on ice for a minimum of 5 minutes. Prepare 400µl of starting dilution (dilu1, 6µg/ml, 3X final concentration) for the Control Ab, Anti-CD20, by adding 24µl of antibody stock solution to 376µl of ADCC Assay Buffer. Store the tube on ice before making antibody serial dilution series.
- **Starting dilutions for two test antibodies:** Decide the starting concentration (1X) for your particular test antibody samples and dilute in ADCC Assay Buffer.

Preparing Antibody Serial Dilutions Using Control Ab, Anti-CD20

1. Obtain a sterile clear V-bottom 96-well plate for preparing antibody threefold serial dilutions for Control Ab, Anti-CD20, and two test antibodies. Add 150µl of Control Ab, Anti-CD20, starting dilution to well A11 and well B11, and dispense 100µl of ADCC Assay Buffer into wells 2–10 within rows A and B. Using a multichannel pipette, transfer 50µl from the antibody starting dilutions in column 11 into column 10. Mix well by pipetting. Avoid creating bubbles. Repeat equivalent threefold serial dilutions across columns from right to left until column 3 is reached.
2. Prepare appropriate serial dilutions specific for each of your test antibodies.

Plating ADCC Bioassay Target Cells (WIL2-S)

1. Dispense 75µl of ADCC Assay Buffer into outermost wells of two white 96-well assay plates, and pre-equilibrate the plate to 37°C.
2. Remove one vial of ADCC Bioassay Target Cells (WIL2-S) from –140°C freezer storage or vapor phase of liquid nitrogen to dry ice for transport to the bench immediately before use. Thaw vial in a 37°C water bath until cells are just thawed (about 2–3 minutes). While thawing, gently agitate and visually inspect. Do not invert.
3. Gently mix the cell suspension by pipetting 1–2 times. Transfer 0.5ml of cells to a tube containing 7.5ml of prewarmed ADCC Assay Buffer. Mix well by gently inverting.
4. Add 25µl of target cells to all wells.
5. Add 25µl per well of antibody dilution series to the wells containing target cells.

Plating ADCC Bioassay Effector Cells

1. Remove 1 vial of ADCC Bioassay Effector Cells from –140°C freezer storage or vapor phase of liquid nitrogen to dry ice for transport to the bench on day of use. Thaw vial in a 37°C water bath until cells are just thawed (about 2–3 minutes). While thawing, gently agitate and visually inspect. Do not invert.
2. Gently mix the cell suspension by pipetting. Transfer 630µl of cells to a tube containing 3.6ml of prewarmed ADCC Assay Buffer. Mix well by gently inverting the tube 2 times.
3. Add 25µl of cells to the wells of the 96-well assay plates already containing target cells and antibody.
4. Cover plates with lids, and incubate the plates for 6 hours at 37°C in a humidified CO_2 incubator.

Adding Bio-Glo™ Luciferase Assay Reagent

1. Remove assay plates from the 37°C incubator and equilibrate to ambient temperature.
2. Add 75µl of Bio-Glo™ Luciferase Assay Reagent to all the assay wells; avoid creating any bubbles.
3. Incubate at ambient temperature for 5–30 minutes.
4. Measure luminescence using the GloMax® Discover System.

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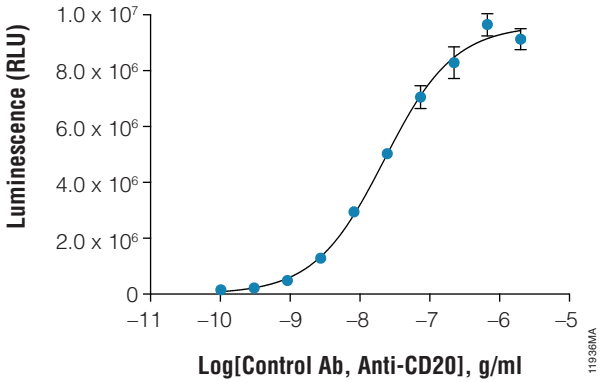

Figure 1. ADCC Reporter Bioassay response to Control Ab, Anti-CD20, using ADCC Bioassay Effector Cells and WIL2-S Target Cells. ADCC Bioassay Target cells were incubated with a series of concentrations of Control Ab, Anti-CD20, followed by addition of ADCC Bioassay Effector Cells. The E:T ratio was 6:1. After 6 hours of induction at 37°C, Bio-Glo™ Luciferase Assay Reagent was added, and luminescence was determined using the GloMax® Discover System. Data shown represents the mean ± standard deviation of six replicates. Data were fitted to a 4PL curve using GraphPad Prism® software. The EC_{50} value determined was 22.4ng/ml.

GloMax® Discover System

The GloMax® Discover System offers superior sensitivity, dynamic range and limited well-to-well cross talk. The instrument has been developed and optimized with Promega's industry-leading cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System also provides flexible use of filters for fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance measurements for adaptation into a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting to your local network.

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