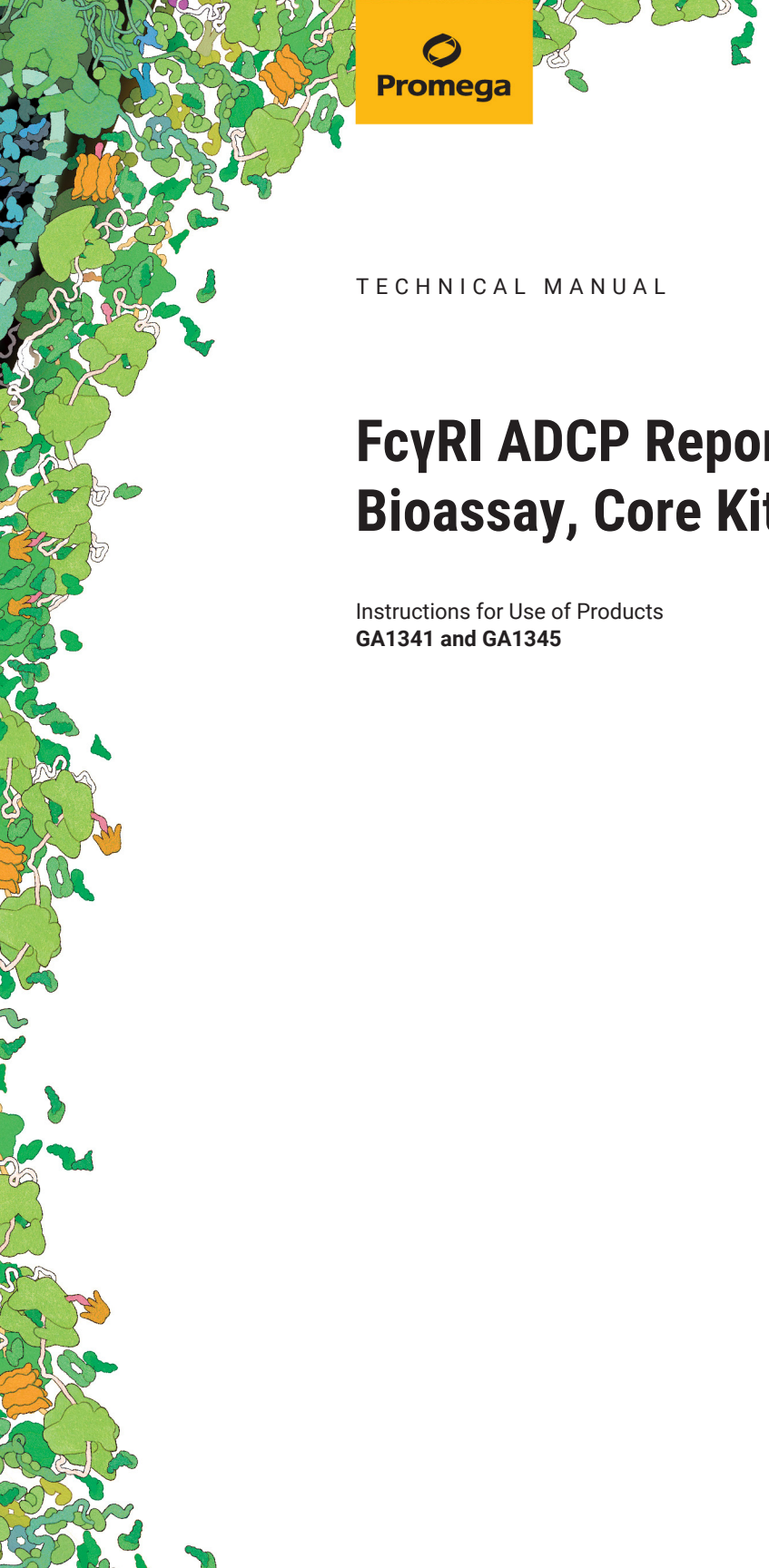

TECHNICAL MANUAL

FcγRI ADCP Reporter Bioassay, Core Kit

Instructions for Use of Products
GA1341 and GA1345

Revised 5/25
TM695

FcγRI ADCP Reporter Bioassay, Core Kit

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Antibody-dependent cell-mediated phagocytosis (ADCP) is an important mechanism of action (MOA) of therapeutic antibodies designed to recognize and mediate the elimination of virus-infected or diseased (e.g., tumor) cells. Unlike antibody-dependent cell-mediated cytotoxicity (ADCC), which is mediated primarily through FcγRIIIa expressed on NK cells, ADCP can be mediated by monocytes, macrophages, neutrophils and dendritic cells via FcγRIIa, FcγRI and FcγRIIIa. In myeloid cells, the expression level of the various receptors is highly dynamic and influenced by cell lineage, tissue microenvironment and local inflammatory state. All three receptors can participate in antibody recognition, receptor clustering and signaling events that result in ADCP (1–5).

Current methods used to measure ADCP rely on the isolation of primary human monocytes, ex vivo differentiation into macrophages and measurement of target cell engulfment. These assays are laborious and highly variable due to their reliance on donor primary cells, complex assay protocols and unqualified assay reagents. As a result, these assays are difficult to establish in quality-controlled, drug development settings.

The FcγRI ADCP Reporter Bioassay, Core Kit[a–d] (Cat.# GA1341, GA1345), is a bioluminescent cell-based assay that overcomes the limitations of existing assays and can be used to measure the potency and stability of antibodies and other biologics with Fc domains that bind and activate FcγRI. The assay consists of a genetically engineered Jurkat T cell line that expresses the high-affinity human FcγRI and a luciferase reporter.

The cell line is provided in a thaw-and-use format, as cryopreserved cells that can be thawed, plated and used in an assay without the need for cell propagation. When cocultured with a target cell and relevant antibody, the FcγRI Effector Cells bind the Fc domain of the antibody, resulting in FcγRI signaling and response element- (RE-) mediated luciferase activity (Figure 1). The bioluminescent signal is detected and quantified using the Bio-Glo™ Luciferase Assay System and a standard luminometer such as the GloMax® Discover System (Cat.# GM3000).

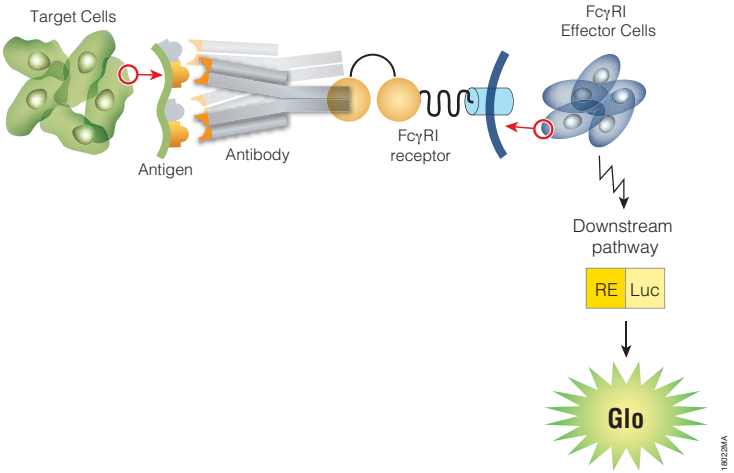

Figure 1. Representation of the FcγRI ADCP Reporter Bioassay. The bioassay consists of a genetically engineered cell line (FcγRI Effector Cells), an antigen expressing target cell and an antigen-specific antibody. When all components are cocultured, the antibody simultaneously binds target cell antigen and FcγRI receptors on the surface of the effector cells. This results in receptor clustering, intracellular signaling and luciferase activity.

The FcγRI ADCP Reporter Bioassay reflects the MOA of biologics designed to bind and activate FcγRI. FcγRI-mediated luminescence is increased in a dose-dependent manner following the addition of Control Ab, Anti-CD20, and Raji Target Cells. Increased luminescence is dependent on the presence of an appropriate antibody and target cell (Figure 2).

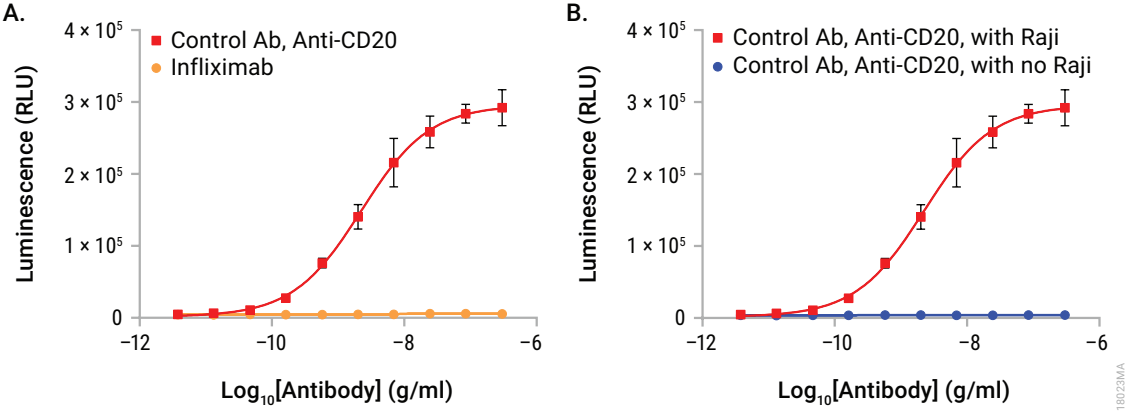

Figure 2. The FcγRI ADCP Reporter Bioassay reflects the MOA and specificity of antibodies designed to bind and activate FcγRI. Panel A. FcγRI ADCP Bioassay Effector Cells were treated with serial dilutions of Control Ab, Anti-CD20 and infliximab (anti-TNFα) in the presence of Raji target cells. **Panel B.** FcγRI ADCP Bioassay Effector Cells were treated with serial dilutions of Control Ab, Anti-CD20, in the presence or absence of Raji target cells. After a 6-hour induction, Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
FcγRI ADCP Reporter Bioassay, Core Kit	**1 each**	**GA1341**

Not for Medical Diagnostic Use. Each kit contains sufficient reagents for 120 assays using the inner 60 wells of two 96-well plates. Includes:

- 1 vial FcγRI ADCP Bioassay Effector Cells (0.7ml)
- 36ml RPMI 1640 Medium
- 4ml Low IgG Serum
- 1 vial Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 10ml Bio-Glo™ Luciferase Assay Buffer

PRODUCT	SIZE	CAT.#
FcγRI ADCP Reporter Bioassay, Core Kit (5X)	**1 each**	**GA1345**

Not for Medical Diagnostic Use. Each kit contains sufficient reagents for 600 assays using the inner 60 wells of ten 96-well plates. Includes:

- 5 vials FcγRI ADCP Bioassay Effector Cells (0.7ml)
- 5 × 36ml RPMI 1640 Medium
- 5 × 4ml Low IgG Serum
- 5 vials Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 5 × 10ml Bio-Glo™ Luciferase Assay Buffer

Storage Conditions:

Upon arrival, immediately transfer the FcγRI ADCP Bioassay Effector Cell vials to below –140°C (freezer or liquid nitrogen vapor phase). Do not store cell vials submerged in liquid nitrogen. **Do not** store cell vials at –80°C because this will negatively affect cell viability and cell performance.

Bio-Glo™ Luciferase Assay Buffer, Bio-Glo™ Luciferase Assay Substrate and Low IgG Serum should be stored at –30°C to –10°C. Avoid multiple freeze-thaw cycles of the serum. For optimal performance, use reconstituted Bio-Glo™ Reagent on the day of preparation. Once reconstituted, Bio-Glo™ Reagent can be stored at –30°C to –10°C for up to 6 weeks.

Store RPMI 1640 Medium at +2°C to +10°C protected from light.

Note: The FcγRI ADCP Reporter Bioassay, Core Kit components are shipped separately because of differing temperature requirements. The FcγRI Effector Cells are shipped on dry ice. The Bio-Glo™ Luciferase Assay System and Low IgG Serum are shipped on dry ice, separately from the cells. The RPMI 1640 Medium is shipped at ambient temperature.

3. Before You Begin

The FcγRI ADCP Reporter Bioassay, Core Kit, differs from classic ADCP assays in a number of ways. Assay parameters including effector:target (E:T) cell ratio, cell number per well, antibody dose range, buffer composition and incubation time may differ from those used in classic ADCP assays using primary macrophages or other cell lines.

Please read through the entire protocol to become familiar with the components and the assay procedure before beginning.

Note the catalog number and lot number from the cell vial box label. This information can be used to download documents for the specified product from the website such as Certificate of Analysis.

The FcγRI ADCP Reporter Bioassay is intended to be used with user-provided antibodies and antigen-expressing target cells. The ADCC Reporter Bioassay, Target Kit (Raji) (Cat.# G7016) is available separately for use in routine quality control. The Target Kit contains Thaw-and-Use CD20+ Raji cells as well as Control Ab, Anti-CD20. We strongly recommend including the ADCC Reporter Bioassay, Target Kit (Raji) as a positive control in the first few assays to gain familiarity with the assay. Data generated using the ADCC Reporter Bioassay, Target Kit (Raji) are shown in Section 8, Representative Assay Results.

The FcγRI ADCP Bioassay Effector Cells are provided in a frozen, thaw-and-use format and are ready to be used without any culturing procedures. When thawed, diluted, and plated as instructed, the cells will be at 75,000 cells/well. The cells are sensitive, and care should be taken to follow cell thawing and plating procedures as described. Do not overmix or overwarm the cell reagents.

The FcγRI ADCP Reporter Bioassay produces a bioluminescent signal and requires a sensitive luminometer or luminescence plate reader for the detection of luciferase activity. Bioassay development and the performance data included in this Technical Manual were generated using the GloMax® Discover System (Cat.# GM3000). An integration time of 0.5 second/well was used for all readings. The bioassay is compatible with most other plate luminometers; however, relative luminescence unit readings may vary due to the sensitivity and settings of each instrument. The use of different instruments and gain adjustments will affect the magnitude of the raw data, but should not affect the measured relative potency.

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3. Before You Begin (continued)

Materials to Be Supplied by the User

- user-defined antibodies
- user-defined target cells
- sterile clear 96-well, V-bottom plate with lid (e.g., Costar® Cat.# 3896)
- solid-white, flat-bottom 96-well assay plates (e.g., Corning® Cat.# 3917) or 384-well assay plates (e.g., Corning® Cat.# 3570) for plating and reading luminescence
- pipettes (single-channel and 12-channel)
- sterile 15ml and 50ml conical tubes
- sterile reagent reservoirs (Corning® Cat.# 4870 or equivalent)
- 37°C, 5% CO_2 incubator
- 37°C water bath
- plate reader with glow luminescence read capability or luminometer (e.g., GloMax® Discover System, Cat.# GM3000)
- **optional:** ADCC Reporter Bioassay, Target Kit (Raji); contains Raji target cells and Control Ab, Anti-CD20, (Cat.# G7016)

4. Preparing Cells, Reagents and Samples

This assay protocol illustrates the use of the of FcγRI ADCP Reporter Bioassay to test two antibody samples against a reference sample in a single assay run. Each test and reference antibody is run in triplicate, in a 10-point dilution series, in a single 96-well assay plate using the inner 60 wells. Other experimental and plate layouts are possible but may require further optimization.

Note: Prior to routine use of the FcγRI ADCP Reporter Bioassay, Core Kit, with your antibody and target cell line, we recommend optimizing the E:T (effector:target cell ratio) and cell densities. When thawed and plated as described in this Technical Manual, the FcγRI Effector Cells will be at 75,000 cells/well. We recommend keeping the effector cell number constant and varying the number of target cells (5,000–25,000 cells/well). For assay optimization, try E:T ratios in the range of 3:1 to 15:1. As a reference, when using Raji target cells and Control Ab, Anti-CD20, we use a 6:1 ratio, with 75,000 effector cells and 12,500 target cells. As a preliminary experiment, this can be further simplified by using a single concentration of antibody and a titration of target cells.

Additional optimization of the antibody dose-range and dilution series may be needed to achieve a full dose-response curve with proper upper and lower asymptotes, and sufficient points throughout the dose range. You can vary the induction time in a range of 6–24 hours to determine the optimal induction time for your antibody. We recommend that you evaluate these parameters rigorously and select the best conditions for your target system.

Notes:

- When using adherent target cells in the FcγRI ADCP Bioassay, target cells are plated the day prior to the assay. When suspension (nonadherent) target cells are used, the target cells are plated the day of the assay.
- For reference, we use 0.3µg/ml of Control Ab, Anti-CD20, as a starting concentration (1X) and 3.5-fold serial dilutions.

4.A. Preparing Bio-Glo™ Reagent, Assay Buffer and Test and Reference Samples

1. **Bio-Glo™ Reagent:** Thaw the Bio-Glo™ Luciferase Assay Buffer at 4°C overnight or in a room temperature water bath on the day of assay. Equilibrate the Bio-Glo™ Luciferase Assay Buffer to ambient temperature, protected from light. Transfer all of the Bio-Glo™ Luciferase Assay Buffer into the amber bottle containing the Bio-Glo™ Luciferase Assay Substrate and mix by inversion until the Substrate is thoroughly dissolved. Equilibrate and store the reconstituted Bio-Glo™ Reagent at ambient temperature (22–25°C) protected from light before adding to the assay plates. When stored appropriately, Bio-Glo™ Reagent will lose 18% activity after 24 hours at ambient temperature. For reference, 10ml of Bio-Glo™ Reagent is sufficient to assay 120 wells in a 96-well assay format.

2. **Assay Buffer:** On the day of assay, thaw the Low IgG Serum in a 37°C water bath until the crystals have just melted. Add 1.5ml of Low IgG Serum to 36ml of RPMI 1640 Medium to make 37.5ml of 96% RPMI 1640/4% Low IgG Serum (sufficient for two assay plates). Mix well and warm to 37°C before use.

   Note: The recommended assay buffer contains 4% Low IgG Serum. This concentration of serum works well for most antibodies and target cells that we have tested. If you experience target cell viability or assay performance issues using this assay buffer, we recommend testing different serum concentrations in the range of 0.5–10%.

3. **Test and Reference Samples:** Prepare starting dilutions (dilu1, 3X final concentration) of two test antibodies (minimum 210µl each) and one reference antibody (minimum of 420µl) using assay buffer as the diluent. Store the tubes containing antibody starting dilutions appropriately before making antibody serial dilutions.

   Notes:

   a. Select starting antibody concentrations based on previous experimental results, if available. Otherwise, use a concentration of 1µg/ml (1X final concentration), then adjust concentration based on assay results.

   b. For reference, if you are using the ADCC Reporter Bioassay, Target Kit (Raji), for testing the assay, prepare 1ml of a starting dilution of 0.9µg/ml (3X) of Control Ab, Anti-CD20, by adding 1.8µl of Control Ab, Anti-CD20, to 998.2µl of assay buffer.

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4.B. Plate Layout Design

For the protocol described here, use the plate layouts illustrated in Figure 3 as a guide. The protocol describes serial replicate dilutions (n = 3) of test and reference antibodies to generate two ten-point dose-response curves in each plate.

Recommended Plate Layout Design													
	1	2	3	4	5	6	7	8	9	10	11	12	
A	B	B	B	B	B	B	B	B	B	B	B	B	Assay Buffer (B)
B	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Reference Ab
C	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Test Ab
D	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Reference Ab
E	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Test Ab
F	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Reference Ab
G	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Test Ab
H	B	B	B	B	B	B	B	B	B	B	B	B	Assay Buffer (B)

Figure 3. Example plate layout showing nonclustered sample locations of test and reference antibody dilution series.

4.C. Plating Target Cells

To prepare the target cells for use with the FcγRI ADCP Reporter Bioassay Core Kit, the cells need to be cultured using standard practices to maintain viability of cells and density in a range satisfactory for good performance in a conventional ADCP assay. Several suspension target cell lines and adherent target cell lines grown in continuous culture have been tested in the FcγRI ADCP Reporter Bioassay and demonstrated good results.

For assay optimization, try different effector-to-target cell (E:T) ratios in the range of 2.5:1 to 25:1. Keep the cell density of FcγRI ADCP Effector Cells constant, and vary the cell density of target cells.

Follow institutional guidelines for handling, including use of personal protective equipment (PPE), and waste disposal for hazardous material.

Perform the following steps in a sterile cell culture hood.

Preparing Adherent Target Cell Lines from Continuous Culture

We recommend passaging the adherent target cells two days before plating for the assay, to ensure optimal and consistent assay performance.

1. Twenty to twenty-four hours before the assay, remove cells from propagation flasks by trypsinization (or other standard procedure), and prepare them for plating in 96-well plates using fresh culture medium.
2. Resuspend cells to an appropriate cell density so that there will be the required cell number for each well (5,000–25,000 cells) in the FcγRI ADCP Bioassay when dispensing 100µl of cells per well.
3. Transfer the cells to a sterile reagent reservoir. Using a multichannel pipette, immediately add 100µl of cells to the inner 60 wells of white 96-well assay plates.
4. Dispense 100µl of culture medium into the outermost wells, labeled "B" in Figure 3, of both assay plates. Place lids on the plates and incubate overnight in a CO_2 incubator at 37°C.
5. On the morning of the assay, invert the assay plate to remove the plating medium. Then, place the inverted plate on paper towels for 5–10 seconds to drain any remaining medium. Alternatively, use a multichannel pipette to remove 95µl of culture medium from each of the wells.
6. Add 25µl per well of assay buffer (prewarmed to 37°C) to the inner 60 wells of both assay plates.
7. Dispense 75µl of assay buffer into the outermost wells, labeled "B" in Figure 3.
8. Cover the plates with lids, and incubate at 37°C, 5% CO_2 while preparing the antibody dilution series.

Preparing Suspension Target Cell Lines from Continuous Culture

We recommend passaging the suspension target cells two days before plating for the assay to ensure optimal and consistent assay performance.

1. On the day of the assay, prepare an appropriate amount of assay buffer as described in Section 4.A. Warm to 37°C.
2. Estimate the quantity of target cells needed, including extra volume to account for reservoir excess.
3. Gently mix and count the target cells by Trypan blue staining.
4. Harvest enough target cells (two to three times the required cell number) by centrifuging at 130 × *g* for 10 minutes.
5. Gently resuspend the cell pellet in warm assay buffer at approximately 2X the target cell density. Count cells by Trypan blue staining and adjust the cell density by adding warm assay buffer to achieve a final cell density of 0.2–1 × 10^6 viable cells/ml (5,000–25,000 cells per 25µl).
6. Transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately add 25µl of cells to the inner 60 wells in both white 96-well assay plates.

4.C. Plating Target Cells (continued)

7. Dispense 75µl of assay buffer into outermost wells, labeled "B" in Figure 3.
8. Cover the plates with lids and incubate at 37°C, 5% CO_2 while preparing antibody dilution series.

Plating Thaw-and-Use ADCC Reporter Bioassay, Target Kit (Raji) Cells:

1. Dispense 75µl of assay buffer into outermost wells, labeled "B" in Figure 3, of two white 96-well assay plates. Incubate the plates at 37°C, CO_2 to pre-equilibrate (10–15 minutes).
2. Label a 15ml conical tube, "Target Cells (Raji)". Add 9.5ml of assay buffer (prewarmed to 37°C) to the tube.
3. Remove one vial of ADCC Reporter Bioassay, Target Kit (Raji) cells from –140°C freezer storage or vapor phase of liquid nitrogen, immediately before use. Place on dry ice for transport to the bench. 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.

   Note: This thawing protocol is recommended and important to the performance of the cells. No further handling is required or recommended.
4. Gently mix the cell suspension by pipetting 1–2 times. Transfer 0.5ml of cells to the tube labeled "Target Cells (Raji)" containing 9.5ml of assay buffer. Mix well by gently inverting the tube two times.
5. Transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately add 25µl of target cells to the inner 60 wells as defined in Figure 3.
6. Cover plates with lids and keep the plates on the bench while preparing antibody dilutions and FcγRI ADCP Bioassay Effector Cells.

4.D. Preparing Antibody Serial Dilutions

These instructions are for preparation of a single stock of 3.5-fold serial dilutions of a single antibody for analysis in triplicate (150µl of each dilution provides a sufficient volume for analysis in triplicate).

Alternatively, you can prepare three independent stocks of serial dilutions to generate triplicate samples. To prepare 3.5-fold serial dilutions, you will need 420µl of reference antibody at 3X the highest antibody concentration for two independent dose-response curves. You will need 210µl of each test antibody at 3X the highest antibody concentration in each of the test antibody dose-response curves. For other dilution schemes, adjust the volumes accordingly.

1. To a sterile clear V-bottom 96-well plate, add 210µl of reference antibody starting dilution (dilu1, 3X final concentration) to wells A11 and B11.
2. Add 210µl of test antibodies 1 and 2 starting dilution (dilu1, 3X final concentration) to wells E11 and G11, respectively.
3. Add 150µl of assay buffer to other wells in these four rows, from column 10 to column 2.
4. Transfer 60µl of the antibody starting dilutions from column 11 to column 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent 3.5-fold serial dilutions across the columns from right to left through column 3. Do not dilute into column 2.

Note: Wells A2, B2, E2 and G2 contain 150µl of assay buffer without antibody as a negative control.

6. Cover the antibody dilution plate with a lid and keep at ambient temperature (22–25°C) while preparing the FcγRI Effector Cells (Section 4.E).

4.E. Preparing FcγRI Effector Cells

1. Label a sterile 15ml conical tube, "FcγRI Effector Cells". Add 3.6ml of assay buffer (prewarmed to 37°C) to the tube.
2. On the day of use, remove one vial of FcγRI ADCP Reporter Bioassay Effector Cells from –140°C freezer storage or vapor phase of liquid nitrogen. Transport to the bench on dry ice. Thaw the 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.

   Note: The recommended thawing protocol in Step 2 is important to the performance of the cells. No further handling is required or recommended.
3. Gently mix the cell suspension by pipetting once or twice. Transfer 630µl of cells to the 15ml "FcγRI Effector Cells" tube containing 3.6ml of assay buffer. Mix well by gently inverting the tube two times.

5. Assay Protocol

5.A. Adding Antibodies and FcγRI Effector Cells to Plates

1. Using a multichannel pipette, add 25µl of the appropriate antibody dilution (prepared in Section 4.D) to the assay plate(s) containing target cells (Section 4.C), according to the plate layout in Figure 3.
2. Transfer the FcγRI ADCP Effector Cells (prepared in Section 4.E) to a sterile reagent reservoir.
3. Using a multichannel pipette, immediately dispense 25µl of the effector cells to each of the inner 60 wells of the assay plates. Gently swirl the assay plates to ensure mixing of the target cells, effector cells and antibody.
4. Cover the assay plates with lids and incubate at 37°C, 5% CO_2 for 6 hours.

5.B. Adding Bio-Glo™ Reagent

Bio-Glo™ Reagent should be at ambient temperature when added to assay plates.

The FcγRI ADCP Bioassay is compatible only with the Bio-Glo™ Luciferase Assay System. Do **not** use the Bio-Glo-NL™ Luciferase Assay System with the FcγRI ADCP Reporter Bioassay, Core Kit.

1. Remove the assay plates from the incubator and equilibrate to ambient temperature (22–25°C) for 15 minutes.
2. Using a manual multichannel pipette, add 75µl of Bio-Glo™ Reagent to the inner 60 wells of the assay plates, taking care not to create bubbles.
3. Add 75µl of Bio-Glo™ Reagent to wells B1, C1, and D1 of each assay plate to measure background signal.
4. Incubate at ambient temperature for 5–30 minutes.

   Note: Varying the incubation time will affect the raw RLU values but should not significantly change the EC_{50} or fold induction.
5. Measure luminescence using a plate reader with glow-type luminescence reading capabilities.

5.C. Data Analysis

1. Determine plate background by calculating the average relative light units (RLU) from wells B1, C1 and D1.
2. Calculate fold induction:

$$\text{Fold Induction} = \frac{\text{RLU (induced} - \text{background)}}{\text{RLU (no antibody control} - \text{background)}}$$

   Note: When calculating fold induction, if the sample RLU are equal to or greater than 100X higher than the plate background RLU, there is no need to subtract the plate background from the sample RLU.
3. Graph data as RLU versus Log_{10} [antibody] and fold induction versus Log_{10} [antibody]. Fit curves and determine EC_{50} of antibody response using appropriate curve fitting software (such as GraphPad Prism® software).

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Weak assay response	Confirm, if known, the antibody affinity to the FcγRI receptor.
	Use the optimal concentration range for the antibody, which provides a full dose response with complete upper and lower asymptotes. Note that the antibody EC_{50} in the FcγRI ADCP Reporter Bioassay will not necessarily be the same as determined from other ADCP bioassays. Thus, some adjustment to the antibody starting concentration and serial dilution schemes may be needed to achieve maximal response in the assay.
	Increase the target cell density while maintaining the effector cell density. Since the readout of the assay is from the effector cells, improvement of the response can be achieved by increasing the number of target cells per well.
	Vary induction times within a 6–24 hour range and choose the induction time that gives the optimal response.
	Verify that the target cells still express antigen at the relevant passage number and method of harvesting.
	Verify that the target cells remain viable and ensure that you are following recommended pre-assay culture directions.
	Optimize the composition of the assay buffer by varying the concentration of low-IgG serum in a range of 0.5–10% and choose the serum concentration that gives the optimal assay response.

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6. Troubleshooting (continued)

Symptoms	Causes and Comments
Poor or low luminescence measurements	Choose a sensitive instrument designed for plate-reading (RLU readout) luminescence detection. Instruments primarily designed for fluorescence are not recommended.
	Luminometers measure and report luminescence as relative values and actual numbers will vary among instruments. Some plate-reading luminometers provide the ability to adjust the photomultiplier tube (PMT) gain to expand the signal range.
	Increase the integration time when reading samples.
	Solid-white assay plates will return the most luminescence; clear-bottom plates will show a significant reduction in luminescence, which can be partially remedied by adding white tape to the bottom of the plate.
Possible issues with matrix effect	IgG, serum complement or other components from serum, supernatant of phage display or hybridoma culture could nonspecifically affect antibody binding to the FcγRI receptor or affect the RE signaling pathway directly, causing a matrix effect. Use low-IgG serum or perform further dilution of antibody starting preparation to minimize its effect.

7. References

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2. Dugast, A.S. *et al.* (2011) Decreased Fc-Receptor expression on innate immune cells is associated with impaired antibody mediated cellular phagocytic activity in chronically HIV-1 infected individuals. *Virology* **415**, 160–7.
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8. Representative Assay Results

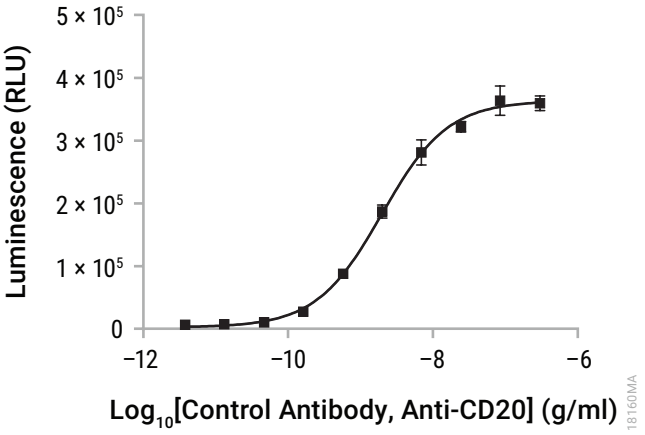

Figure 4. The FcγRI ADCP Reporter Bioassay with Raji Target Cells measures the activity of Control Ab, Anti-CD20. On the day of assay, Raji Target Cells, FcγRI Effector Cells and a titration of Control Ab, Anti-CD20, were added as described in this technical manual. After a 6-induction at 37°C, Bio-Glo™ Reagent was added and luminescence measured using a GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. The EC_{50} was 1.72ng/ml and fold induction was 65. Data were generated using thaw-and-use cells.

9. Summary of Changes

The following changes were made to the 5/25 revision of this document:

1. Revised text about the label in Section 3.
2. Updated fonts.
3. Made miscellaneous text edits.

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