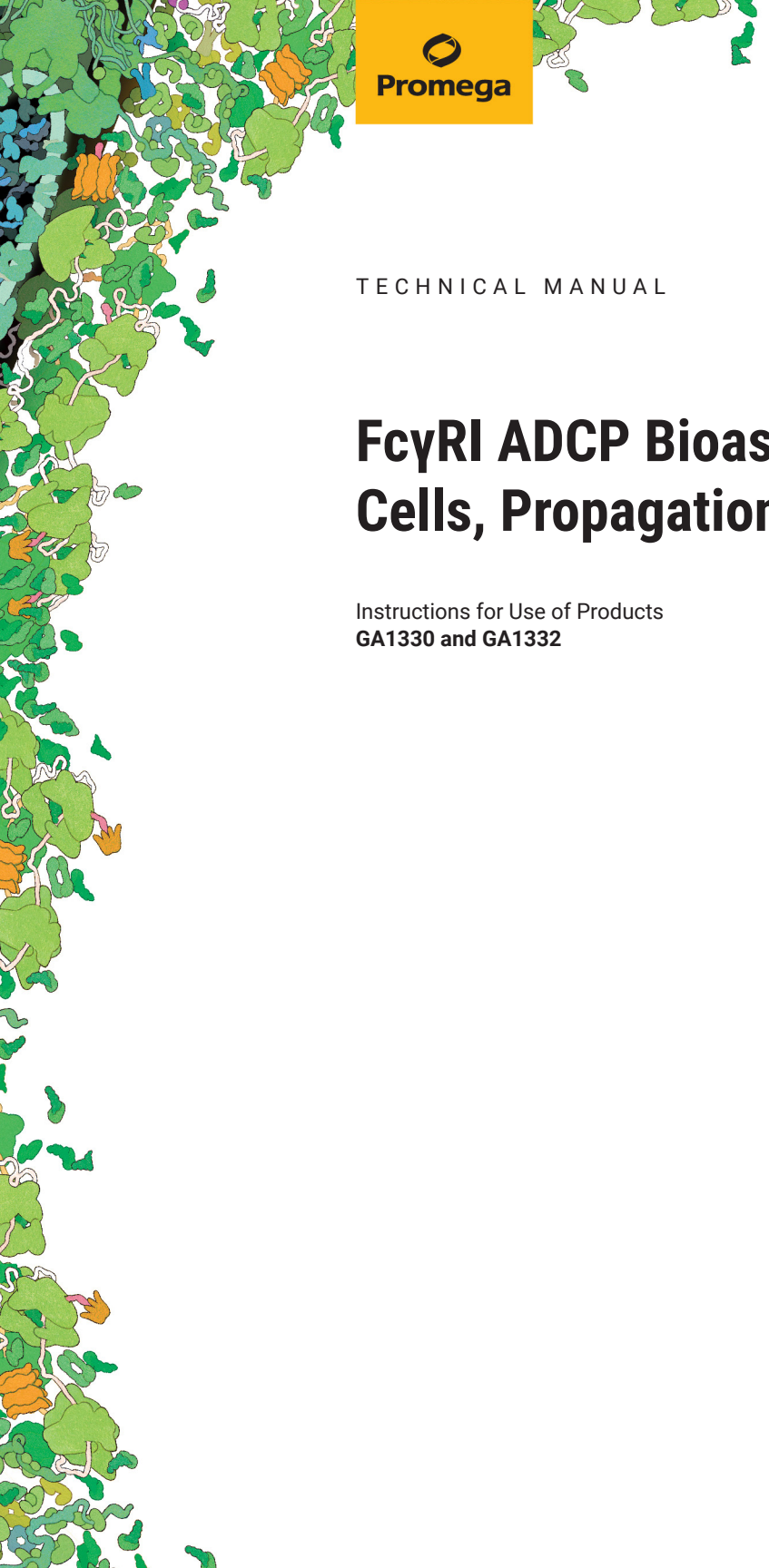

TECHNICAL MANUAL

# FcγRI ADCP Bioassay Effector Cells, Propagation Model

Instructions for Use of Products
**GA1330 and GA1332**

Revised 5/25
TM692

# FcγRI ADCP Bioassay Effector Cells, Propagation Model

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**Visit the website to verify that you are using the most current version of this Technical Manual.**
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## 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 Bioassay Effector Cells, Propagation Model[a–c] (Cat.# GA1330, GA1332), 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 cell propagation model (CPM) and cell bank formats, which include cryopreserved cells that can be thawed, propagated and banked for long-term use. 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).

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).

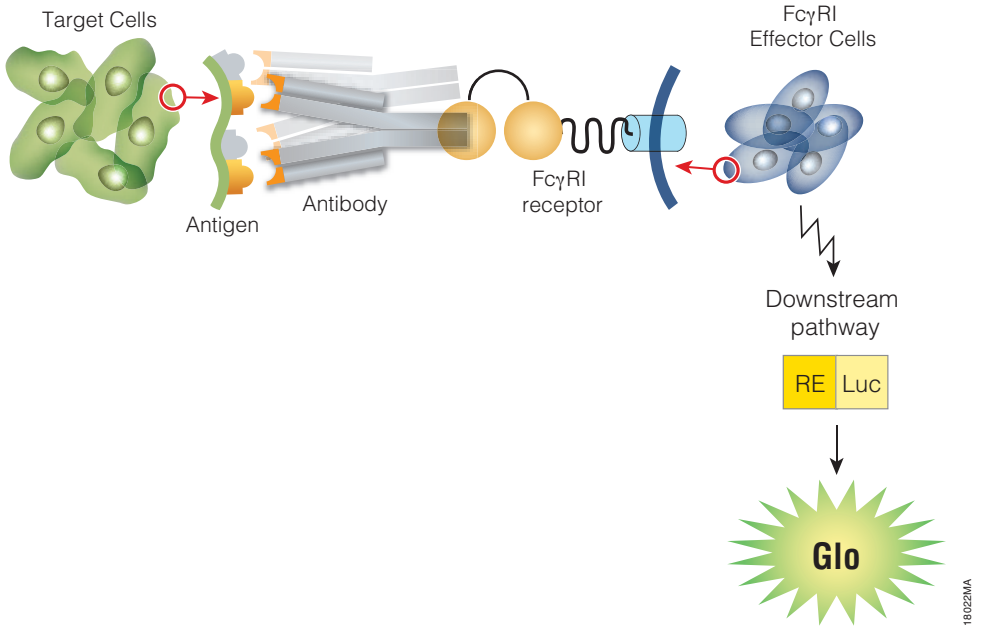


**Figure 1. Representation of the FcγRI ADCP Reporter Bioassay.** The bioassay consists of a genetically engineered cell line (FcγRI Effector Cells), an antigen expressing target cell and an antigen-specific antibody. When all components are cocultured, the antibody simultaneously binds target cell antigen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

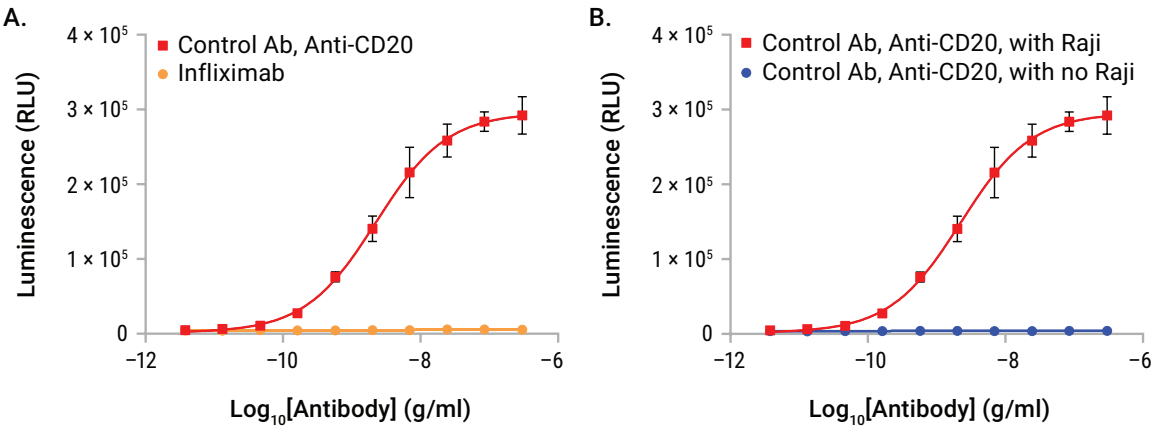


**Figure 2. The FcγRI ADCP Reporter Bioassay reflects the MOA and specificity of antibodies designed to bind and activate FcγRI. Panel A.** FcγRI ADCP Bioassay Effector Cells were treated with serial dilutions of Control Ab, Anti-CD20 and infliximab (anti-TNFα) in the presence of Raji target cells. **Panel 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 Bioassay Effector Cells, Propagation Model** | **1 each** | **GA1332** |

Not for Medical Diagnostic Use. Includes:

- 2 vials FcγRI Effector Cells (CPM), 2 × $10^7$ cells/ml (1.0ml per vial)

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **FcγRI ADCP Bioassay Effector Cells, Cell Bank** | **1 each** | **GA1330** |

Not for Medical Diagnostic Use. Includes:

- 50 vials FcγRI Effector Cells (CPM), 2 × $10^7$ cells/ml (1.0ml per vial)

**Storage Conditions:** Upon arrival, immediately transfer the 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.

## 3. Before You Begin

The FcγRI ADCP Reporter Bioassay 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.**

Cell thawing, propagation and banking should be performed exactly as described in Section 4. Cell seeding and propagation densities have been optimized to ensure stable cell growth, which is reflected in a steady cell doubling rate to achieve optimal and consistent performance. By contrast, the recommended cell plating densities, induction time and assay buffer components described in Section 4 were established using a small panel of antibodies that activate FcγRI. You may need to adjust these parameters and optimize assay conditions for your own antibodies or other biologic samples.

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 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. An integration time of 0.5 seconds/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.

### Materials to Be Supplied by the User

#### Reagents

- user-defined antibodies
- user-defined target cells
- RPMI 1640 with L-glutamine and HEPES (GIBCO® Cat.# 22400)
- fetal bovine serum (FBS; e.g., GIBCO® Cat.# 35-015-CV or Cytiva HyClone Cat.# SH30071.03)
- super low IgG FBS (Cytiva HyClone Cat.# SH30898.02)
- DPBS (GIBCO® Cat.# 14190)
- hygromycin (GIBCO® Cat.# 10687-010)
- G418 Geneticin® (e.g., GIBCO® Cat.# 10131035)
- Accutase® solution (e.g., Sigma Cat.# A6964)
- DMSO (Sigma Cat.# D2650)
- Trypan blue solution (Sigma Cat.# T8154)
- Bio-Glo™ Luciferase Assay System (Cat.# G7940 or Cat.# G7941)
- **optional:** ADCC Reporter Bioassay, Target Kit (Raji); contains Raji target cells and Control Ab, Anti-CD20; (Cat.# G7016)

## 3. Before You Begin (continued)

### Supplies and Equipment

- sterile clear 96-well, V-bottom plate with lid (e.g., Costar® Cat.# 3896) for preparing antibody dilutions
- 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 $CO_2$ incubator
- 37°C water bath
- plate reader with glow luminescence read capability or luminometer (e.g., GloMax® Discover System, Cat.# GM3000)
- cryogenic vials

## 4. Preparing FcγRI ADCP Effector Cells

**Table 1. Quick Guide to Medium Compositions.**

| | Cell Thawing Medium | Cell Growth Medium | Cell Freezing Medium |
|---|---|---|---|
| RPMI 1640 | 90% | 90% | 85% |
| FBS | 10% | 10% | 10% |
| Hygromycin | | 100µg/ml | |
| Geneticin | | 250µg/ml | |
| DMSO | | | 5% |

### 4.A. Cell Thawing and Initial Cell Culture

1. Prepare 25ml of cell thawing medium (Table 1) and warm to 37°C. Add 9ml of this medium to a new 50ml conical tube.
2. Remove one vial of FcγRI Effector Cells from storage at –140°C and thaw in a 37°C water bath with gentle agitation (do not mix by inversion) until just thawed, typically 2–3 minutes.
3. Transfer all the cells (approximately 1ml) into the 50ml conical tube containing 9ml of prewarmed cell thawing medium.
4. Centrifuge at 130 × *g* for 10 minutes.
5. Carefully aspirate the medium and resuspend the cell pellet in 15ml of prewarmed cell thawing medium.
6. Transfer the 15ml of FcγRI Effector Cell suspension to a T75 tissue culture flask. Place flask horizontally in a humidified 37°C, 5% $CO_2$ incubator.
7. Incubate the cells for 2–3 days. Count cells and monitor cell density until it reaches 1.2–1.8 × $10^6$ cells/ml.

### 4.B. Cell Maintenance and Propagation of FcγRI ADCP Effector Cells

For cell maintenance and propagation starting from the second cell passage, use the cell growth medium containing hygromycin and Geneticin® (Table 1) and monitor cell viability and doubling rate during propagation. The cell growth rate will stabilize by 7–10 days post-thaw, at which time cell viability is typically >95% and the average cell doubling rate is 24–36 hours. Passage number should be recorded for each passage. Cells will maintain their functionality for 25 passages (or 58 doublings if passaging is performed on a Monday-Wednesday-Friday schedule). Cells should be banked appropriately soon after growth rate stabilization.

1. Determine cell density in the flask after 2–3 days in culture, and include Trypan blue staining during counting to monitor cell viability.
2. Seed the cells at a density of $4 \times 10^5$ viable cells/ml if passaging every two days (e.g., Monday–Wednesday, Wednesday–Friday) or $2.5 \times 10^5$ viable cells/ml if passaging every three days (e.g., Friday–Monday) by adding fresh cell growth medium to the cell suspension in the original flask or by transferring the cells to a new flask. Maintain a consistent ratio of culture volume to flask surface area (e.g., 20–30ml volume per T75cm flask or 40–60ml volume per T150cm flask).
3. Place the flasks horizontally in a 37°C, 5% $CO_2$ incubator.

   **Note:** Changing culture volumes, seeding densities or propagation density range may affect cell growth rate and performance of the cells in the assay.

### 4.C. Cell Freezing and Banking of FcγRI ADCP Effector Cells

1. On the day of cell freezing, prepare new cell freezing medium and keep it on ice.
2. Gently mix the cells with a pipette to create a homogeneous cell suspension.
3. Remove a sample for cell counting by Trypan blue staining. Calculate the volume of cell freezing medium needed based on desired cell freezing density between $4 \times 10^6$–$2 \times 10^7$cells/ml.
4. Transfer the cell suspension to 50ml sterile conical tubes or larger size centrifuge tubes and centrifuge at 130 × *g* for 10 minutes.
5. Gently aspirate the medium, taking care not to disturb the cell pellet.
6. Carefully resuspend the cell pellet in ice-cold cell freezing medium to a final cell density of $4 \times 10^6$–$2 \times 10^7$cells/ml. Combine the cell suspension into a single tube and dispense into cryovials.
7. Freeze the cells using a controlled-rate freezer (preferred), or in a suitable freezing container (e.g., Mr. Frosty® or a Styrofoam® rack) in a –80°C freezer. Transfer the vials to below –140°C or into the vapor phase of liquid nitrogen for long-term storage.

## 5. Assay Protocol

This assay protocol illustrates the use of the of FcγRI ADCP Effector Cells, Propagation Model, 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 ten-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 Bioassay Effector Cells, Propagation Model with your antibody and target cell line, we recommend optimizing the E:T (Effector:Target cell ratio) and cell densities. Fix the number of FcγRI Effector Cells at 75,000 cells/well for a 96-well plate and vary the number of target cells (5,000–25,000 cells/well for a 96-well plate). This will help establish an E:T ratio and cell density that give a strong signal response and fold induction. For assay optimization, try E:T ratios in the range of 2.5:1 to 25:1. 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 dilution.

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

1. **Bio-Glo™ Reagent:** Prepare Bio-Glo™ Reagent according to the manufacturer's instructions. For reference, 10ml of Bio-Glo™ Reagent is sufficient for 120 wells in a 96-well assay format. Thaw the Bio-Glo™ Luciferase Assay Buffer in a refrigerator 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 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 the reconstituted Bio-Glo™ Reagent to ambient temperature before adding to assay plates.

   If you are using a large (100ml) Bio-Glo™ Luciferase Assay System, you can dispense the reconstituted Bio-Glo™ Reagent into 10ml aliquots and store at –20°C for up to six weeks. Avoid repeated freeze-thaw cycles. On the day of the assay, thaw the appropriate amount of reconstituted Bio-Glo™ Reagent in a room temperature water bath for at least 1–2 hours before use. Bio-Glo™ Reagent can be stored at room temperature after reconstitution with ~18% loss in luminescence after 24 hours or at 4°C with ~12% loss of luminescence after 5 days.

2. **Assay Buffer:** Prepare an appropriate amount of assay buffer on the day of assay. Thaw the low-IgG FBS in a 37°C water bath, taking care not to overheat it, and add an appropriate amount to RPMI 1640 medium to yield 96% RPMI 1640/4% low-IgG FBS. Mix well and warm to 37°C before use. For reference, 40ml of assay buffer is typically sufficient for 120 wells in a 96-well assay format using the inner 60 wells.

   **Note:** The recommended assay buffer contains 4% super low IgG FBS. This concentration of FBS 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 (1X final concentration) based on previous experimental results, if available. Otherwise, we recommend starting with a concentration of 1µg/ml (1X), which has worked well for antibodies we have tested.

   b. If you are using the 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.

### 5.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.**

### 5.C. Preparing and Plating Adherent Target Cells the Day Before Assay

Target cells for use in the FcγRI ADCP Reporter Bioassay should be maintained in culture according to established protocols for each individual cell type. Cell viability, antigen expression and assay reproducibility require that the target cells are cultured within an optimal cell density range and window of passage stability. We recommend passaging the target cells two days before plating for the assay to ensure optimal and consistent assay performance. Both adherent and suspension target cells have been used successfully with the FcγRI ADCP Reporter Bioassay.

Perform the following steps in a sterile cell culture hood.

1. We recommend passaging the Adherent Target Cells two days before plating for the assay to ensure optimal and consistent assay performance.
2. Aspirate the cell culture medium from the adherent target cells and wash with DPBS.

3. Add 2ml of Accutase® solution to each T75 flask, and place the flask in a 37°C, 5% $CO_2$ incubator for 5–7 minutes or until the cells round up and detach from the bottom of the flask.
4. Add 8ml of antibiotic-free medium to the flask. Transfer the cell suspension to a 50ml (or larger) conical centrifuge tube.
5. Gently mix and count the adherent target cells by Trypan blue staining.
6. Centrifuge at 130 × *g* for 10 minutes.
7. Gently resuspend the cell pellet in antibiotic-free medium to achieve a concentration of 0.5–2.5 × $10^5$ viable cells/ml (5,000–25,000 cells per 100µl).
8. Transfer the suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 100µl of the cell suspension to each of the inner 60 wells of a 96-well white flat-bottom assay plate.
9. Add 100µl of antibiotic-free medium to each of the outside wells of the assay plates.
10. Place lids on the assay plates and place in a 37°C, 5% $CO_2$ incubator overnight (18–24 hours).

### 5.D. Preparing Suspension Target Cells the Day of Assay

Target cells for use in the FcγRI ADCP Reporter Bioassay should be maintained in culture according to established protocols for each individual cell type. Cell viability, antigen expression and assay reproducibility require that the target cells are cultured within an optimal cell density range and window of passage stability.

1. We recommend passaging the suspension target cells two days before plating for the assay to ensure optimal and consistent assay performance.
2. On the day of the assay, prepare an appropriate amount of assay buffer as described in Section 5.A. Warm to 37°C.
3. Estimate the quantity of target cells needed, including extra volume to account for reservoir excess.
4. Gently mix and count the target cells by Trypan blue staining.
5. Harvest the required number of cells by centrifuging in a conical tube for 10 minutes at 130 × *g*.
6. 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).
7. Incubate the target cell suspension at 37°C to maintain the temperature while preparing effector cells and antibody dilution series.

### 5.E. Preparing Antibody Serial Dilutions

The instructions described here 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 into 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.

### 5.F. Preparing FcγRI Effector Cells

While maintaining the FcγRI Effector Cells, follow the recommended cell seeding density as changes in cell culture volume or seeding density may affect the cell growth rate and assay performance. Only use the cells after the cell doubling rate has stabilized during propagation. Do not use cells that have poor viability or that have exceeded the maximum recommended density of $1.8 \times 10^6$ cells/ml.

1. Passage the cells 2 days before performing the assay as described in Section 4.B. To ensure optimal and consistent assay performance, maintain the cell density, upon harvest, in the range of $1.0–1.6 \times 10^6$ cells/ml and cell viability at greater than 95%.
2. Count the FcγRI Effector Cells by Trypan Blue staining and calculate the cell density and viability.
3. Transfer an appropriate amount of FcγRI Effector Cells from the culture vessel to a 50ml conical tube or larger-sized centrifuge tube.
4. Pellet the cells at 130 × *g* for 10 minutes at ambient temperature and resuspend in assay buffer at 70% of full volume needed to generate a cell suspension at $3 \times 10^6$ cells/ml, based on the cell counts determined in Step 2.
5. Count the cells again, and adjust the volume of assay buffer to achieve a final cell density of $3 \times 10^6$ cells/ml. You will need at least 4ml of FcγRI Effector Cells to fill the inner 60 wells of two assay plates (3ml plus excess for pipettor and reservoir loss).
6. Incubate the FcγRI Effector Cells at 37°C to maintain the temperature if plating suspension target cells.

### 5.G. Plating Suspension Target Cells, Antibodies and FcγRI Effector Cells

1. Gently mix the previously prepared target cell suspension (Section 5.D).
2. Add the target cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 25µl of the cells to each of the inner 60 wells of a 96-well flat-bottom, white, assay plate.
3. Using a multichannel pipette, add 25µl of the appropriate antibody dilution to the plated target cells, according to the plate layout in Figure 3.
4. Mix the FcγRI Effector Cells (Section 5.F) by inverting the tube, and transfer the suspension to a sterile reagent reservoir.
5. Using a multichannel pipette, immediately dispense 25µl of the 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.
6. Add 75µl of assay medium to each of the outside wells of the assay plates.
7. Cover the assay plates with lids and incubate the plate in a 37°C, 5% $CO_2$ incubator for 6–24 hours.

### 5.H. Adding Antibodies and FcγRI Effector Cells to Preplated Adherent Target Cells

1. Take the 96-well assay plates containing adherent target cells out of the incubator. Invert the assay plate above a sink to remove the medium. Then place the inverted plate on paper towels for 5–10 seconds to drain any remaining medium. Alternatively, remove 95µl of medium from each of the wells using a manual multichannel pipette.
2. Using a multichannel pipette, add 25µl of assay buffer to the inner 60 wells of both 96-well assay plates.
3. Using a multichannel pipette, add 25µl of the appropriate antibody dilution to the plated target cells, according to the plate layout in Figure 3.
4. Mix the FcγRI Effector Cells (prepared in Section 5.F) by inverting the tube, then transfer the suspension to a sterile reagent reservoir.
5. Using a multichannel pipette, immediately dispense 25µl of the FcγRI Effector Cells to each of the inner 60 wells of the assay plates already containing antibody and target cells to yield 75,000 FcγRI Effector Cells per well.
6. Cover the assay plates with lids and incubate the plate in a 37°C, 5% $CO_2$ incubator for 6–24 hours.

### 5.I. 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 Bioassay.

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.J. 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 |
|---|---|
| Poor viability of FcγRI Effector Cells during cell culture | Confirm that the serum and antibiotic concentrations are correct. Ensure that all reagents are within their indicated expiration dates. |
| | Confirm that incubator temperatures and $CO_2$ levels are correctly set. |
| | Confirm the passage cell density ($2.5 \times 10^5$cells/ml if passaging every 3 days or $4 \times 10^5$ cells/ml if passaging every 2 days). Confirm previous passage harvest density. |
| Weak assay response | Confirm, if known, the antibody affinity to the FcγRI receptor. |
| | Make sure to use the optimal concentration range for the antibody, which can provide 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. |
| | Increase the FcγRI Effector Cell density together with an increase in target cell density. |
| | Vary induction times within a range of 6–24 hours 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. |
| | Verify the viability and density of the FcγRI Effector Cells at harvesting (Section 5.F). |
| | Optimize the composition of the assay buffer by varying the concentration of low-IgG FBS 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 FBS or perform further dilution of antibody starting preparation to minimize impact. |

## 7. References


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## 8. Representative Assay Results

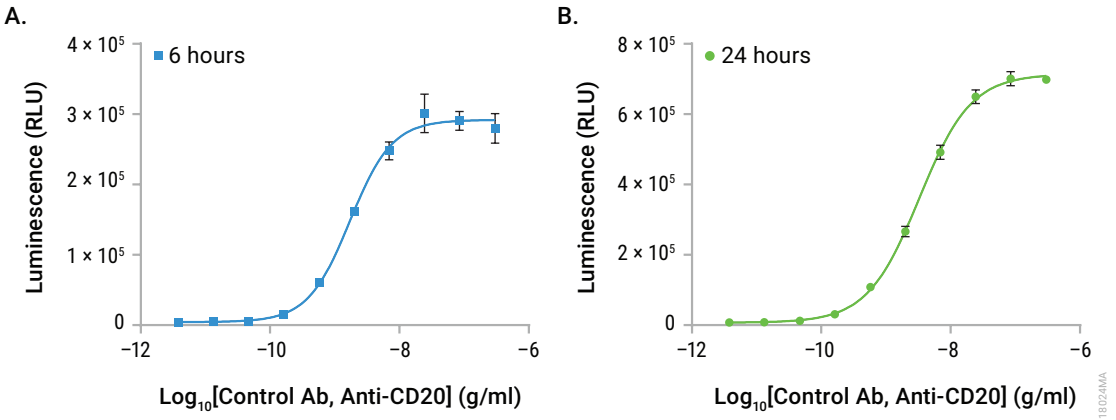


**Figure 4. The FcγRI ADCP Reporter Bioassay with Raji Target Cells measures the activity of Control Ab, Anti-CD20.** On the day of assay, Raji Target Cells, FcγRI Effector Cells and a titration of Control Ab, Anti-CD20 were added as described in this technical manual. After a 6-hour (**Panel A**) or a 24-hour induction (**Panel B**) 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 3.27ng/ml, for 6- and 24-hour inductions, respectively, and fold induction was 65 and 90.4, for 6- and 24-hour inductions, respectively. Data were generated using cell propagation model cells.

## 9. Summary of Changes

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

1. Removed an expired patent statement.
2. Revised text about the label in Section 3.
3. Updated the fonts.

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