

TECHNICAL MANUAL

# Fc $\gamma$ RIIa-H ADCP Reporter Bioassay, Complete Kit

Instructions for Use of Product  
**G9901**



# Fc $\gamma$ RIIa-H ADCP Reporter Bioassay, Complete Kit

All technical literature is available at: [www.promega.com/protocols/](http://www.promega.com/protocols/)  
 Visit the web site to verify that you are using the most current version of this Technical Manual.  
 E-mail Promega Technical Services if you have questions on use of this system: [techserv@promega.com](mailto:techserv@promega.com)

1. Description.....	2
2. Product Components and Storage Conditions .....	7
3. Before You Begin.....	7
4. Assay Protocol .....	9
4.A. Preparing Bio-Glo™ Reagent, Assay Buffer, and Test and Control Ab Samples .....	9
4.B. Plate Layout Design .....	10
4.C. Preparing and Plating Target Cells .....	11
4.D. Preparing Antibody Serial Dilutions.....	11
4.E. Adding Antibodies to Plated Target Cells .....	12
4.F. Preparing and Plating Fc $\gamma$ RIIa-H Effector Cells .....	12
4.G. Adding Bio-Glo™ Reagent .....	13
4.H. Data Analysis .....	13
5. Troubleshooting.....	14
6. References.....	16
7. Related Products.....	16

## 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 Fc $\gamma$ RIIIa expressed on NK cells, ADCP can be mediated by monocytes, macrophages, neutrophils and dendritic cells via Fc $\gamma$ RIIa (CD32a), Fc $\gamma$ RI (CD64), and Fc $\gamma$ RIIIa (CD16a). In macrophages, 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, immune receptor clustering, and signaling events that result in ADCP; however, blocking studies suggest that Fc $\gamma$ RIIa is the predominant Fc $\gamma$ R receptor involved in this process (1-4).

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.

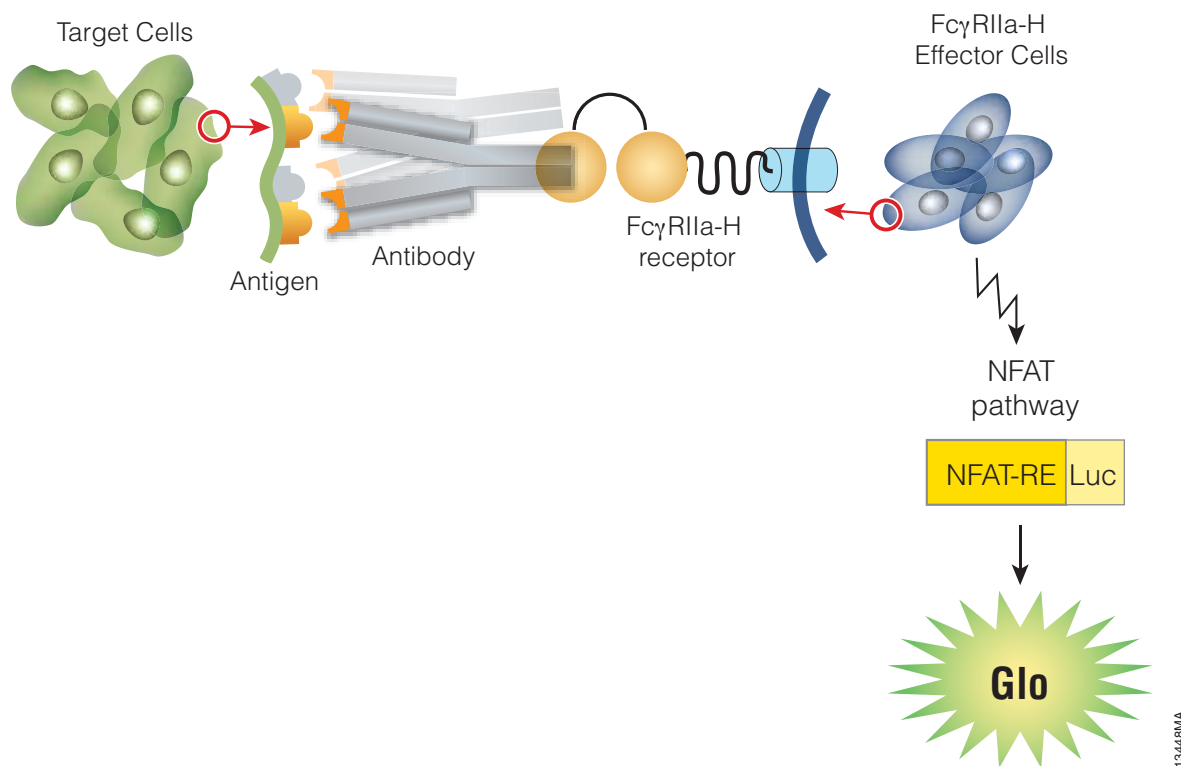
The Fc $\gamma$ RIIa-H ADCP Reporter Bioassay, Complete Kit<sup>(a-d)</sup> (Cat.# G9901), is a bioluminescent cell-based assay that overcomes the limitations of existing assays and can be used to measure the potency and stability of antibodies and other biologics with Fc domains that specifically bind and activate Fc $\gamma$ RIIa. The assay consists of a genetically engineered Jurkat T cell line that expresses:

- The high-affinity human Fc $\gamma$ RIIa-H variant that contains a Histidine (H) at amino acid 131
- A luciferase reporter driven by an NFAT-response element (NFAT-RE)

Compared to the low-affinity Fc $\gamma$ RIIa-R variant that contains an arginine (R) at amino acid 131, Fc $\gamma$ RIIa-H exhibits higher affinity for IgG2 isotypes. The Fc $\gamma$ RIIa-H Effector Cells are provided in a thaw-and-use format, which includes cryopreserved cells that can be thawed, plated and used in an assay without the need for propagation.

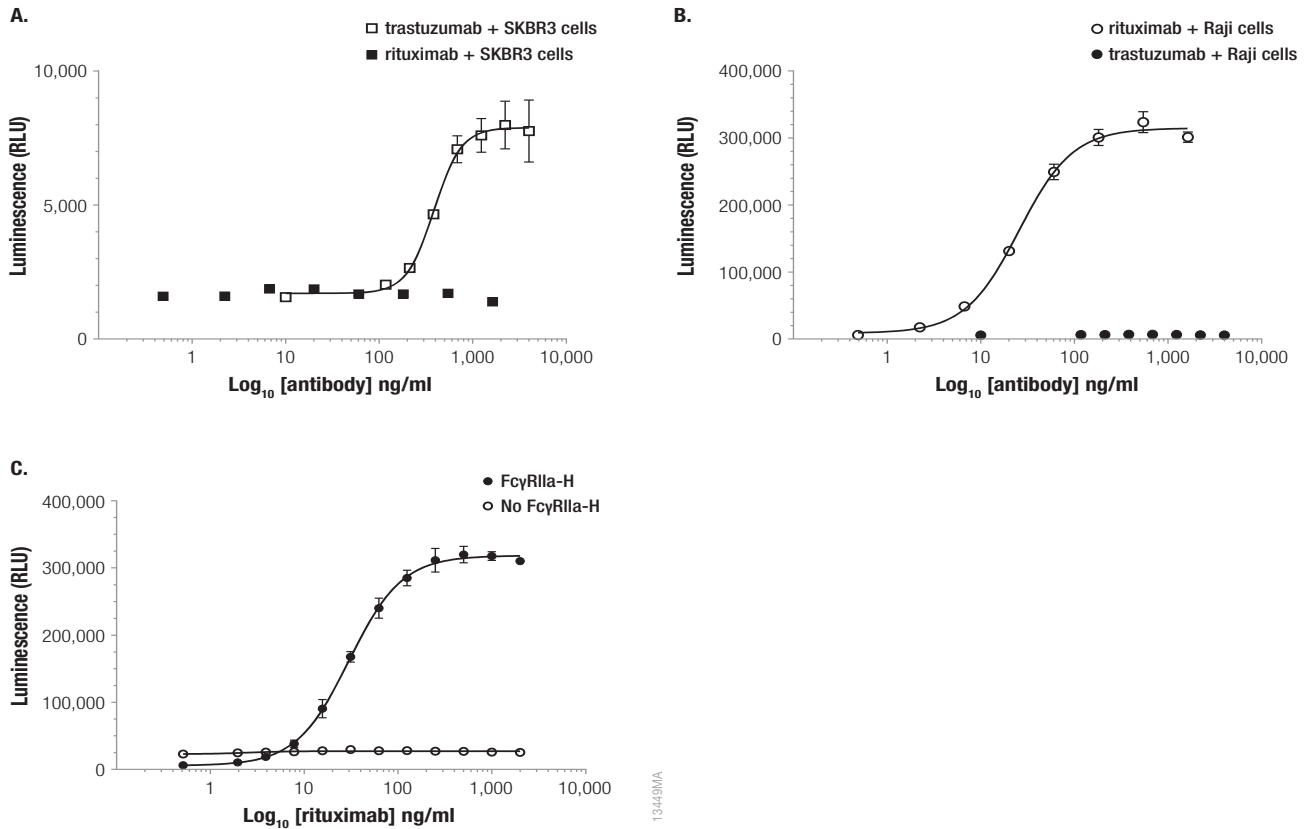
When co-cultured with a target disease cell and relevant antibody, the Fc $\gamma$ RIIa-H Effector Cells bind the Fc domain of the antibody, resulting in Fc $\gamma$ RIIa signaling and NFAT-RE-mediated luciferase activity (Figure 1). The bioluminescent signal is detected and quantified using Bio-Glo™ Luciferase Assay System (Cat.# G7940, G7941) and a standard luminometer such as the GloMax® Discover System (see Related Products, Section 7).

The assay combines (1) a simple, add-mix-read single-day workflow with (2) Fc $\gamma$ RIIa-H Effector Cells and Target Cells (Raji) provided in a frozen, thaw-and-use format, and (3) an optimized protocol that together yield a quantitative bioassay that exhibits low variability and high accuracy. The thaw-and-use cells provided in the Fc $\gamma$ RIIa-H ADCP Reporter Bioassay kits are manufactured under stringent quality control to provide high assay reproducibility with the convenience of an assay reagent that eliminates the need for continuous cell propagation.

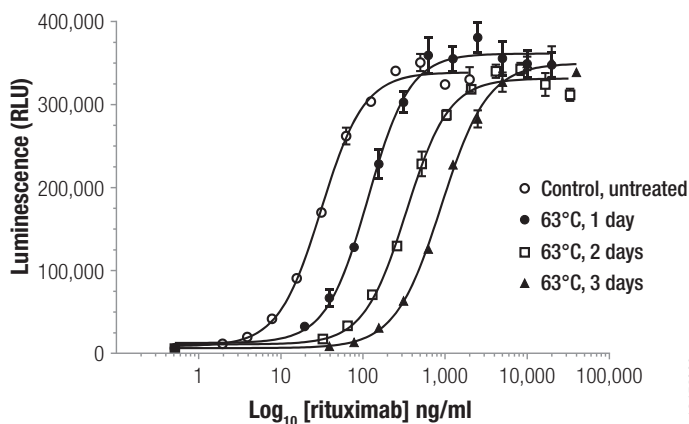


**Figure 1. Representation of the Fc $\gamma$ RIIa-H ADCP Reporter Bioassay.** The bioassay consists of a genetically engineered cell line (Fc $\gamma$ RIIa-H Effector Cells), an antigen expressing target cell and an antigen-specific antibody. When all components are co-cultured, the antibody simultaneously binds the target cell antigen and Fc $\gamma$ RIIa-H receptors on the surface of the effector cells. This results in receptor clustering, intracellular signaling and NFAT-RE-mediated luciferase activity.

The Fc $\gamma$ RIIa-H ADCP Reporter Bioassay reflects the MOA of biologics designed to bind and activate Fc $\gamma$ RIIa. The bioassay shows high specificity as demonstrated using trastuzumab (anti-HER2) or rituximab (anti-CD20) antibodies and the relevant target cells SKBR3 (HER2<sup>+</sup>) or Raji (CD20<sup>+</sup>), respectively (Figure 2). In response to trastuzumab, Fc $\gamma$ RIIa-mediated luciferase activity is detected using SKBR3 target cells, but not Raji cells. Conversely, rituximab-induced luciferase activity is detected using Raji target cells, but not SKBR3 cells. No luciferase activity is detected using NFAT-RE effector cells that do not express Fc $\gamma$ RIIa. The bioassay shows precision, accuracy and linearity required for potential validation and routine use in potency and stability studies (Figure 3 and Table 1). Finally, the Fc $\gamma$ RIIa-H ADCP Reporter Bioassay is compatible with both adherent and non-adherent target cells (Figure 4).



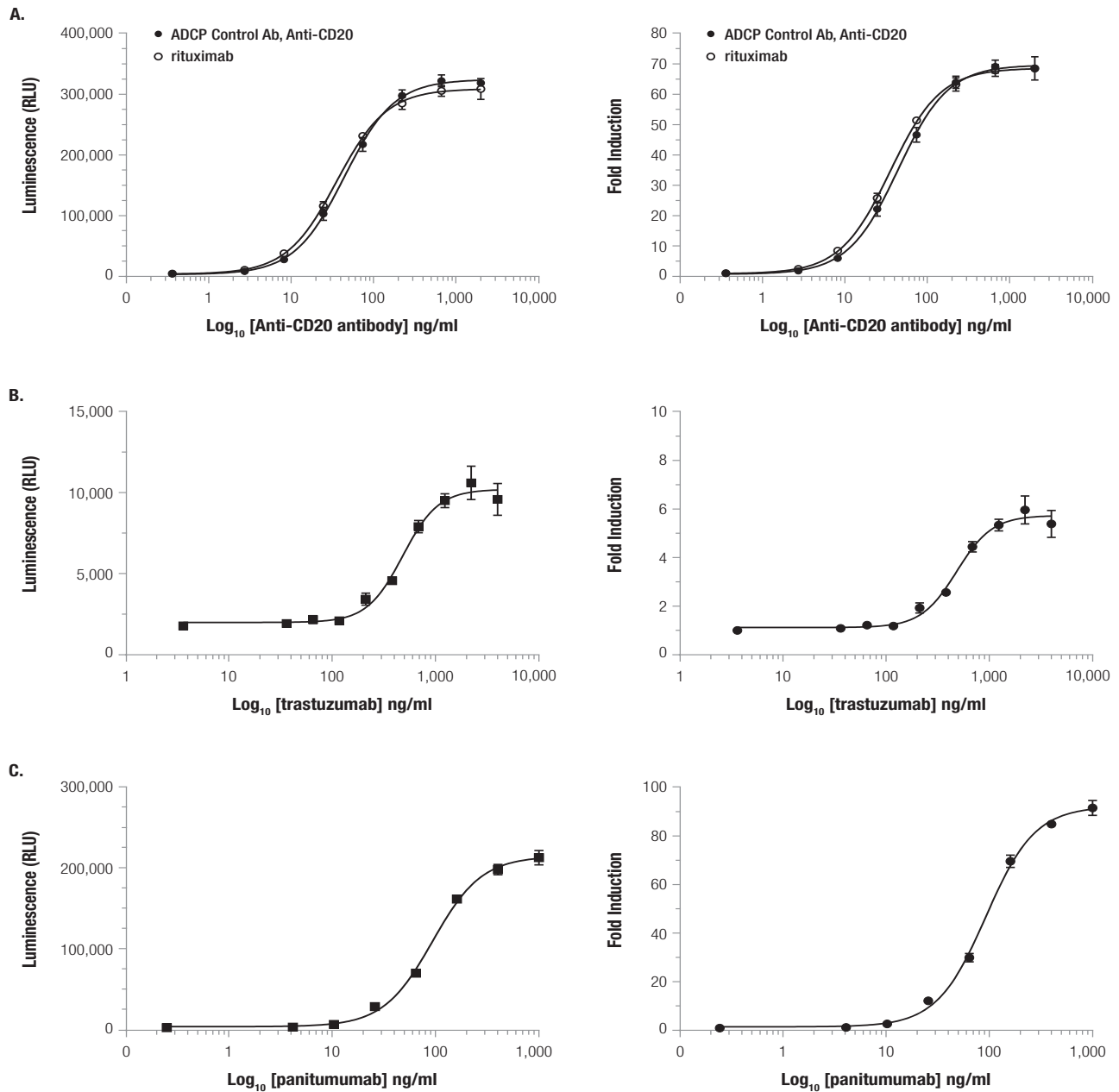
**Figure 2. The Fc $\gamma$ RIIa-H ADCP Reporter Bioassay reflects the MOA and specificity of antibodies designed to bind and activate Fc $\gamma$ RIIa-H. Panels A and B.** Increasing concentrations of trastuzumab (anti-HER2) or rituximab (anti-CD20) were incubated with either SKBR3 (HER2<sup>+</sup>) or Raji (CD20<sup>+</sup>) target cells and Fc $\gamma$ RIIa-H Effector Cells, as indicated. **Panel C.** Increasing concentrations of rituximab were incubated with Raji target cells and NFAT-RE effector cells either with or without Fc $\gamma$ RIIa-H expression. Bio-Glo™ Reagent was added, and luminescence was measured. Data were fitted to a 4PL curve using GraphPad Prism® software. With the exception of SKBR3 cells, data were generated using thaw-and-use cells.



**Figure 3. The Fc $\gamma$ RIIa-H ADCP Reporter Bioassay is stability-indicating.** Samples of rituximab (anti-CD20) were maintained at 4°C (control) or heat denatured at 63°C for the indicated times and analyzed using the Fc $\gamma$ RIIa-H ADCP Reporter Bioassay. The EC<sub>50</sub> values were 32ng/ml (control) and 116ng/ml, 339ng/ml, and 904ng/ml across the three time points. Data were fitted to a 4PL curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

**Table 1. The Fc $\gamma$ RIIa-H ADCP Reporter Bioassay Demonstrates Accuracy, Repeatability, Intermediate Precision and Linearity.**

Parameter	Results	
	% Expected Relative Potency	% Recovery
Accuracy	50	99.1
	71	102.7
	140	105.0
	200	99.1
Repeatability (% CV)	100% reference of 3 days	3.5
Intermediate Precision (% CV)		6.5
Linearity (r <sup>2</sup> )		0.997
Linearity (y = mx + b)		y = 0.997x + 1.95
A 50–200% theoretical relative potency series of the ADCP Control Ab, anti-CD20 (IgG1), was analyzed in triplicate in three independent experiments performed on three days. Luciferase activity was quantified using the Bio-Glo™ Reagent. Data were analyzed and relative potencies calculated after parallelism determination using JMP® software. Data were generated using thaw-and-use cells.		



**Figure 4. The FcγRIIa-H ADCP Reporter Bioassay is compatible with both adherent and non-adherent target cells. Panel A.** Increasing concentrations of anti-CD20 antibody (ADCP Control Ab, Anti-CD20 or rituximab, both IgG1, as indicated) **Panel B.** Trastuzumab (anti-HER2, IgG1) or **(Panel C)** panitumumab (anti-EGFR, IgG2) were incubated with either Raji (CD20<sup>+</sup>, non-adherent, Panel A) or SKBR3 (HER2<sup>+</sup>, adherent, Panel B) or A431 (EGFR<sup>+</sup>, adherent, Panel C) target cells and FcγRIIa-H Effector Cells. Bio-Glo™ Reagent was added, and luminescence was measured. Data were fitted to a 4PL curve using GraphPad Prism® software. With the exception of SKBR3 and A431 cells, data were generated using thaw-and-use cells. All data were obtained following a 6-hour induction.

## 2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
<b>FcγRIIa-H ADCP Reporter Bioassay, Complete Kit</b>	<b>1 each</b>	<b>G9901</b>

Not for Medical Diagnostic Use. Includes:

- 1 vial FcγRIIa-H Effector Cells (0.62ml)
- 1 vial Target Cells (Raji) (0.55ml)
- 1 vial ADCP Control Ab, Anti-CD20 (lyophilized, 2.5μg)
- 36ml RPMI 1640 Medium
- 4ml Low IgG Serum
- 1 vial Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 10ml Bio-Glo™ Luciferase Assay Buffer



**Note:** The FcγRIIa-H ADCP Reporter Bioassay components are shipped separately because of temperature requirements. The FcγRIIa-H Effector Cells and Target Cells (Raji) are shipped on dry ice. The Bio-Glo™ Luciferase Assay System, Low IgG Serum and ADCP Control Ab, Anti-CD20, are shipped on dry ice, separately from the cells. The RPMI 1640 Medium is shipped at ambient temperature.

### Storage Conditions:

- Upon arrival, immediately transfer the cell vials to below –140°C (freezer or liquid nitrogen vapor phase) for long-term storage. 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.
- Store ADCP Control Ab, Anti-CD20, Bio-Glo™ Luciferase Assay Substrate, Bio-Glo™ Luciferase Assay Buffer and Low IgG Serum at –20°C. Avoid multiple freeze-thaw cycles of the serum.
- For optimal performance, use reconstituted Bio-Glo™ Luciferase Assay Reagent only on the day of preparation. You may store reconstituted, Bio-Glo™ Luciferase Assay Reagent at –20°C for up to 6 weeks.
- Store RPMI 1640 Medium at 4°C protected from fluorescent light.

## 3. Before You Begin

The FcγRIIa-H ADCP Reporter Bioassay, Complete Kit, is intended to be used as a starter kit to gain familiarity with the FcγRIIa-H Reporter Bioassay, and for this purpose you should use the FcγRIIa-H Effector Cells, Target Cells (Raji) and ADCP Control Ab, Anti-CD20, provided with the kit. If your target antigen is CD20 or another antigen expressed on Raji Cells, you may analyze your own test and reference antibodies using the cell components provided with this kit. To analyze test and reference antibodies with a different target cell, refer to instructions and guidelines provided with the FcγRIIa-H ADCP Reporter Bioassay, Core Kit (Cat.# G9991, G9995).



The Fc $\gamma$ RIIa-H 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.** The Fc $\gamma$ RIIa-H Effector Cells and Target Cells (Raji) are provided in frozen, thaw-and-use format and are ready to be used without any additional cell culture or propagation. When thawed and diluted as instructed, the cells will be at the appropriate concentration for the assay. The cells are sensitive, and care should be taken to follow the cell thawing and plating procedures *exactly* as described in Section 4, taking care not to over mix or over-warm the cell reagents.

The Fc $\gamma$ RIIa-H 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<sup>®</sup>-Multi Detection System. 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.

#### **Materials to Be Supplied by the User**

- user-defined test antibodies or derivatives with Fc effector function
- sterile, clear 96-well plate with lid (e.g., Costar #3370 or Linbro Cat. #76-223-05)
- white, flat-bottom 96-well assay plates (e.g., Corning Cat.# 3917)
- sterile 15ml and 50ml conical tubes
- sterile water (used to reconstitute the ADCP Control Ab, Anti-CD20)
- sterile reagent reservoirs (e.g., Corning Cat.# 4870)
- 37°C, 5% CO<sub>2</sub> incubator
- 37°C water bath
- plate reader with glow luminescence measuring capability or luminometer (e.g., GloMax<sup>®</sup> Discover System)

## 4. Assay Protocol

This assay protocol illustrates the use of the Fc $\gamma$ RIIa-H ADCP Reporter Bioassay to test two antibody samples against a control sample (ADCP Control Ab, Anti-CD20) in a single assay run. Each test and control 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 Fc $\gamma$ RIIa-H ADCP Reporter Bioassay with your own antibody and target cell lines, we recommend optimizing the Effector:Target cell (E:T) ratio and cell densities. Fix the number of Fc $\gamma$ RIIa-H Effector Cells (20,000–50,000 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. As a preliminary experiment, this can be simplified by using a single concentration of antibody (e.g., 2–5 $\mu$ g/ml). 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. Induction times of 6–18 hours are a good starting point for the assay. You can vary the induction time further to determine an optimal or convenient time. We recommend that you evaluate these parameters rigorously and select the best conditions for your target system.

### 4.A. Preparing Bio-Glo™ Reagent, Assay Buffer, and Test and Control Ab Samples

1. **Bio-Glo™ Reagent:** Prepare Bio-Glo™ Reagent according to the manufacturer's instructions. For your 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. Approximate stability of Bio-Glo™ Reagent after reconstitution is 18% loss of luminescence over 24 hours at ambient temperature.
2. **Assay Buffer:** On the day of assay, monitor and 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%.

#### 4.A. Preparing Bio-Glo™ Reagent, Assay Buffer and Test and Control Ab Samples (continued)

3. **Test Samples:** Using assay buffer as the diluent, prepare starting dilutions (dilu1, 3X final concentration; see Figure 5) of two test antibodies (minimum 200µl each) in 1.5ml tubes. Store the tubes containing antibody starting dilutions appropriately before making antibody serial dilutions.

**Note:** Select starting antibody concentrations (1X final concentration) based on previous experimental results, if available. Otherwise, we recommend starting with a concentration of 1–5µg/ml, which has worked well for rituximab, panitumumab and trastuzumab in the FcγRIIa-H ADCP Reporter Bioassay.

4. **ADCP Control Ab, Anti-CD20, Sample:** Remove the ADCP Control Ab, Anti-CD20, from the freezer and ensure that the lyophilized antibody sample is at the bottom of the vial. Add 50µl of water to the bottom of the vial and incubate it at room temperature 2–5 minutes. Next, add 366µl of assay buffer to the vial and replace the cap. If lyophilized material is visible along the sides or cap of the vial, carefully tilt and roll the vial to rehydrated the material. Mix gently by pipetting, being careful to avoid creating bubbles. Place the reconstituted control antibody at 4°C until ready to use (Section 4.D).

**Note:** The total volume is 416µl, with the ADCP Control Ab, Anti-CD20, at 6µg/ml (dilu1, 3X final concentration).

#### 4.B. Plate Layout Design

For the protocol described here, use the plate layout illustrated in Figure 5 as a guide. The protocol describes serial replicate dilutions (n = 3) of test and reference antibodies to generate two 10-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	Control 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	Control 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	Control 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 5. Example plate layout showing non-clustered sample locations of the ADCP Control Ab, Anti-CD20, dilution series and a single test antibody dilution series.**

#### 4.C. Preparing and Plating Target Cells

**Note:** The thaw-and-use Target Cells (Raji) included in this kit are sensitive and care should be taken to follow the cell thawing and plating procedures **exactly** as described taking care not to over mix or over warm the cell reagents. No additional cell culture or manipulation is required or recommended. We recommend thawing and diluting a maximum of 2 vials of thaw-and-use cells at any one time.

**Note:** To analyze antibodies or derivatives with a different target cell, refer to instructions and guidelines provided with the Fc $\gamma$ RIIa-H ADCP Reporter Bioassay, Core Kit (Cat.# G9991, G9995).

1. Add 7.8ml of prewarmed (37°C) assay buffer to a 15ml conical tube.
2. Remove one vial of Target Cells (Raji) from storage at –140°C and transfer to the bench on dry ice.
3. Thaw the cells in a 37°C water bath with gentle agitation (no inversion) until just thawed (about 2 minutes).
4. Immediately mix the cell suspension by pipetting gently 3 times. Transfer 0.5ml of the cell suspension into the 15ml tube containing prewarmed assay buffer. Immediately mix by inversion several times.
5. Transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 25 $\mu$ l of the cell suspension to each of the inner 60 wells of a 96-well, white, flat-bottom assay plate.
6. Add 75 $\mu$ l of assay buffer to each of the outside wells of the assay plates.
7. Equilibrate the target cells for approximately 15 minutes in a 37°C, 5% CO<sub>2</sub> incubator.

#### 4.D. Preparing Antibody Serial Dilutions

The instructions described here are for preparation of a single stock of 2.5-fold serial dilutions of a single antibody for analysis in triplicate (120 $\mu$ l 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 2.5-fold serial dilutions, you will need 400 $\mu$ l of ADCP Control Ab, Anti-CD20, antibody at 3X the highest concentration for each dose-response curve (already prepared in Section 4.A). You will need 200 $\mu$ l of each test antibody at 3X the highest concentration in each of the test-antibody dose-response curves. For other dilution schemes, adjust the volumes accordingly. The Fc $\gamma$ RIIa-H ADCP Reporter Bioassay works well with rituximab and panitumumab using 2.5-fold serial dilutions at 0–2 $\mu$ g/ml final concentration.

1. Using a sterile clear 96-well plate, add 200 $\mu$ l of ADCP Control Ab, Anti-CD20, starting dilution (dilu1, 3X final concentration) to wells A11 and B11.
2. Add 200 $\mu$ l of test antibodies 1 and 2 starting dilution (dilu1, 3X final concentration) to wells C11 and D11, respectively (see Figure 6).
3. Add 120 $\mu$ l of assay buffer to other wells in these four rows, from column 10 to column 2.
4. Transfer 80 $\mu$ l of the antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent 2.5-fold serial dilutions across the columns from right to left, through column 3. Do not dilute into column 2.



**Note:** Wells A2, B2, C2 and D2 contain 120 $\mu$ l of assay buffer without antibody as a negative control.

#### 4.D. Preparing Antibody Serial Dilutions (continued)

Recommended Plate Layout for Antibody Dilutions Prepared from a Single Antibody Stock													
	1	2	3	4	5	6	7	8	9	10	11	12	
A		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Control Ab
B		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Control Ab
C		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Test Ab 1
D		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Test Ab 2
E													
F													
G													
H													

**Figure 6. Example plate layout showing antibody serial dilutions.**

#### 4.E. Adding Antibodies to Plated Target Cells

- Using a multichannel pipette, add 25µl of the appropriate antibody dilution to the pre-plated target cells according to the plate layout in Figure 5.
- Cover the assay plate with a lid and incubate it in a 37°C incubator for 15 minutes.

#### 4.F. Preparing and Plating FcγRIIa-H Effector Cells


**Note:** The thaw-and-use FcγRIIa-H Effector Cells included in this kit are sensitive and care should be taken to follow the cell thawing and plating procedures exactly as described taking care not to over mix or over-warm the cell reagents. No additional cell culture or manipulation is required or recommended. We recommend thawing and diluting a maximum of 2 vials of thaw-and-use cells at any one time.

- Add 5.3ml of prewarmed (37°C) assay buffer to a 15ml conical tube.
- Remove one vial of FcγRIIa-H Effector Cells from storage at -140°C and transfer to the bench on dry ice.
- Thaw the cells in a 37°C water bath with gentle agitation (no inversion) until just thawed (about 2 minutes).
- Immediately mix the cell suspension by pipetting gently 3 times. Transfer 0.55ml of the cell suspension into the 15ml tube containing prewarmed assay buffer. Immediately mix by inversion several times.
- Hold briefly at 37°C or transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately add 25µl of the cell suspension to each of the wells of the 96-well plates already containing target cells and antibody.
- Cover the assay plates with a lid and incubate in a 37°C, 5% CO<sub>2</sub> incubator for 6 hours. Do not stack the plates inside the incubator.

#### 4.G. Adding Bio-Glo™ Reagent

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


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 impact the raw RLU values but should not significantly change the EC<sub>50</sub> and fold induction.

5. Measure luminescence using a luminometer or luminescence plate reader.

#### 4.H. Data Analysis

1. Measure plate background by calculating the average relative light units (RLU) from wells B1, C1 and D1.
2. Calculate fold induction = RLU (induced – background) / RLU (no antibody control – background).

 **Note:** When calculating fold induction, if the sample RLUs 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<sub>10</sub> [antibody] and fold induction versus Log<sub>10</sub> [antibody]. Fit curves, and determine EC<sub>50</sub> of antibody response using appropriate curve fitting software (such as GraphPad Prism® software).

## 5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: [www.promega.com](http://www.promega.com). E-mail: [techserv@promega.com](mailto:techserv@promega.com)

<u>Symptoms</u>	<u>Possible Causes and Comments</u>
Weak assay response	<p data-bbox="803 457 1508 535">Confirm, if known, the antibody affinity to the Fc<math>\gamma</math>RIIa receptor.</p> <hr/> <p data-bbox="803 546 1508 850">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<sub>50</sub> in the Fc<math>\gamma</math>RIIa-H 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.</p> <hr/> <p data-bbox="803 861 1508 1018">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.</p> <hr/> <p data-bbox="803 1029 1508 1102">Increase the Fc<math>\gamma</math>RIIa-H Effector Cell density together with an increase in target cell density.</p> <hr/> <p data-bbox="803 1113 1508 1186">Vary induction times within a range of 4–24 hours, and choose the induction time that gives the optimal response.</p> <hr/> <p data-bbox="803 1197 1508 1270">If applicable, verify that the target cells still express antigen at the relevant passage number and method of harvesting.</p> <hr/> <p data-bbox="803 1281 1508 1396">If applicable, verify that the target cells remain viable, and ensure that you are following recommended pre-assay culture directions.</p> <hr/> <p data-bbox="803 1407 1508 1568">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.</p>



**Symptoms**

**Possible Causes and Comments**

Poor or low luminescence measurements (RLU readout)

Choose a sensitive instrument designed for plate-reading 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 Fc $\gamma$ RIIa receptor or affect the NFAT-RE signaling pathway directly, causing a matrix effect. Use low-IgG FBS or perform further dilution of antibody starting preparation to minimize impact.

Will I see the same ranking of antibody potency in the Promega Fc $\gamma$ RIIa-H ADCP Reporter Bioassay as in a classic ADCP bioassay?

The Fc $\gamma$ RIIa-H ADCP Reporter Bioassay will measure antibody Fc-mediated signaling specifically through the Fc $\gamma$ RIIa-H receptor, which data suggest is the primary Fc receptor through which antibodies mediate ADCP in vivo (1–4). However, Fc $\gamma$ RIIIa and Fc $\gamma$ RI may also contribute to ADCP function in vivo, and those receptors are not represented in the Fc $\gamma$ RIIa-H ADCP Reporter Bioassay.



## 6. References

1. Richards, J.O. *et al.* (2008) Optimization of antibody binding to FcγRIIa enhances macrophage phagocytosis of tumor cells. *Mol. Cancer Ther.* **7**, 2517–27.
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.
3. Ackerman, M.E. *et al.* (2013) Enhanced phagocytic activity of HIV-specific antibodies correlates with natural production of immunoglobulins with skewed affinity for FcγR2a and FcγR2b. *J. Virol.* **87**, 5468–76.
4. Tebo, A.E. *et al.* (2002) Fcγ receptor-mediated phagocytosis of *Plasmodium falciparum*-infected erythrocytes *in vitro*. *Clin. Exp. Immunology* **130**, 300–6.

## 7. Related Products

### Fc Effector Bioassays

Product	Size	Cat. #
FcγRIIa-H ADCP Reporter Bioassay, Core Kit <sup>1</sup>	1 each	G9991
FcγRIIa-H ADCP Reporter Bioassay, Core Kit, 5X <sup>1</sup>	1 each	G9995
ADCC Reporter Bioassay, Complete Kit (Raji) <sup>1</sup>	1 each	G7015
ADCC Reporter Bioassay, Target Kit (Raji) <sup>1</sup>	1 each	G7016
ADCC Reporter Bioassay, F Variant, Core Kit <sup>2</sup>	1 each	G9790

<sup>1</sup>Not For Medical Diagnostic Use. Additional kit formats are available.

<sup>2</sup>For Research Use Only. Not for Use in Diagnostic Procedures. Additional kit formats are available.

### Immunotherapy Bioassays

Product	Size	Cat. #
PD-1/PD-L1 Blockade Bioassay	1 each	J1250
PD-1/PD-L1 Blockade Bioassay, 5X	1 each	J1255
Control Ab, Anti-PD-1	1 each	J1201
PD-L1 Negative Cells	1 each	J1191
PD-L1 Negative Cells, 5X	1 each	J1195

Not for Medical Diagnostic Use. Additional kit formats are available.

**Detection Reagents**

<b>Product</b>	<b>Size</b>	<b>Cat.#</b>
Bio-Glo™ Luciferase Assay System	10ml	G7941
	100ml	G7940

Not For Medical Diagnostic Use.

**Luminometers**

<b>Product</b>	<b>Size</b>	<b>Cat.#</b>
GloMax® Discover System	1 each	GM3000

For Research Use Only. Not for Use in Diagnostic Procedures.

**Note:** Additional Fc Effector and immunotherapy bioassays are available from Promega Custom Assay Services. To view and order products from Custom Assay Services visit: [www.promega.com/CAS](http://www.promega.com/CAS) or e-mail: [CAS@promega.com](mailto:CAS@promega.com)



<sup>(a)</sup> NOT FOR MEDICAL DIAGNOSTIC USE. FOR IN VITRO USE ONLY. BY USE OF THIS PRODUCT, RECIPIENT AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE STATEMENT. If the recipient is not willing to accept the conditions of this limited use statement, and the product is unused, Promega will accept return of the unused product and provide the recipient with a full refund.

This product may not be further sold or transferred by the recipient and may be used only by the recipient, and then only for (1) research use, (2) drug discovery and development of biologic drugs, (3) quality assurance testing of biologic drugs, and (4) product release assays for biologic drugs. No other commercial use is allowed. "Commercial use" means any and all uses of this product by recipient for monetary or other consideration, including providing a service, information or data to unaffiliated third parties, and resale of this product for any use. Recipient has no right to propagate, modify, derivatize, genetically engineer or otherwise create variations of the Effector Cells or the luciferase gene stably transfected within the Effector Cells. In addition, recipient must use Bio-Glo™ Luciferase Assay System purchased from Promega Corporation for all determinations of luminescence activity of this product, or contact Promega to obtain a license for use of this product with reagents other than Promega's. PROMEGA MAKES NO REPRESENTATIONS OR WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED, INCLUDING AS TO MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE WITH REGARDS TO THIS PRODUCT. The terms of this agreement shall be governed under the laws of the State of Wisconsin, USA.

<sup>(b)</sup> NOT FOR MEDICAL DIAGNOSTIC USE. FOR IN VITRO USE ONLY. BY USE OF THIS PRODUCT, RECIPIENT AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE STATEMENT. If the recipient is not willing to accept the conditions of this limited use statement, and the product is unused, Promega will accept return of the unused product and provide the recipient with a full refund.

This product may not be further sold or transferred by the recipient and may be used only by the recipient, and then only for (1) research use, (2) drug discovery and development of biologic drugs, (3) quality assurance testing of biologic drugs, and (4) product release assays for biologic drugs. No other commercial use is allowed. "Commercial use" means any and all uses of this product by recipient for monetary or other consideration, including providing a service, information or data to unaffiliated third parties, and resale of this product for any use. Recipient has no right to propagate, modify, derivatize, genetically engineer or otherwise create variations of the Target Cells. In addition, recipient must use Bio-Glo™ Luciferase Assay System purchased from Promega Corporation for all luminescence assays using this product, or contact Promega to obtain a license for use of this product with reagents other than Promega's. PROMEGA MAKES NO REPRESENTATIONS OR WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED, INCLUDING AS TO MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE WITH REGARDS TO THIS PRODUCT. The terms of this agreement shall be governed under the laws of the State of Wisconsin, USA.

<sup>(c)</sup>U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

<sup>(d)</sup>Licensed from Lonza Cologne GmbH under U.S. Pat. Nos. 7,700,357, 8,192,990 and 8,003,389, European Pat. Nos. 1297119, 1522587, 1607484 and 1741778 and other pending and issued patents.

© 2016 Promega Corporation. All Rights Reserved.

GloMax is a registered trademarks of Promega Corporation. Bio-Glo is a trademark of Promega Corporation.

GraphPad Prism is a registered trademark of GraphPad Software, Inc. JMP is a registered trademark of SAS Institute Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products