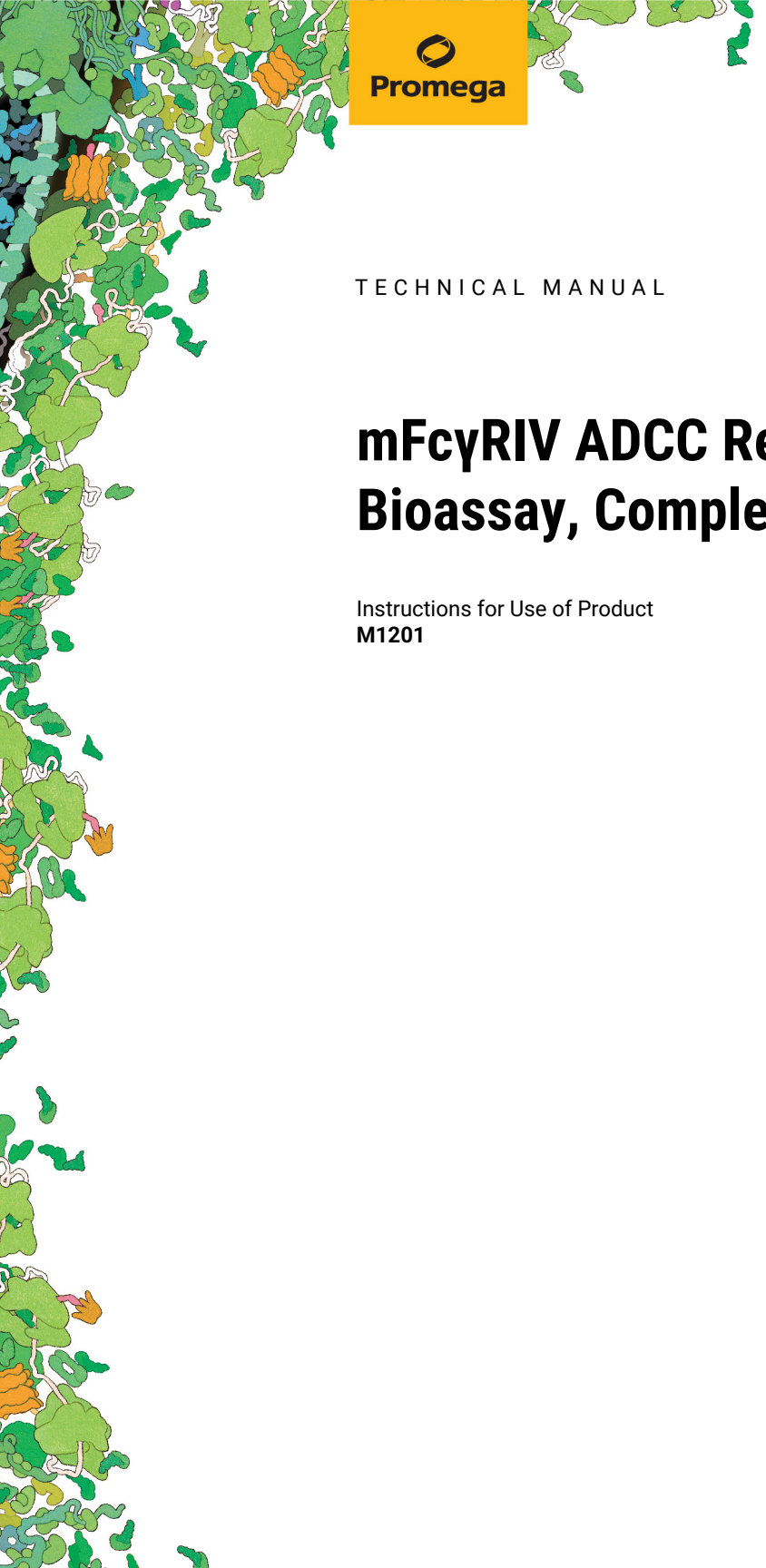


TECHNICAL MANUAL

mFcγRIV ADCC Reporter Bioassay, Complete Kit

Instructions for Use of Product
M1201

Revised 10/26
TM485

mFcγRIV ADCC Reporter Bioassay, Complete 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

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1. Description

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an important mechanism of action (MOA) of antibodies designed that target virus-infected or diseased (e.g., tumor) cells for destruction by components of the cell-mediated immune system. Mouse FcγRIV (mFcγRIV) is the predominant receptor involved in ADCC in the mouse and is more closely related to human FcγRIIIa, the primary Fc receptor involved in ADCC in humans, than mFcγRIII. Mouse IgG2a, and to a lesser extent IgG2b, are known to mediate ADCC through the activation of mFcγRIV. In contrast, mouse IgG1 does not bind to mFcγRIV. In vivo, mouse IgG1-mediated ADCC activity is inhibited via binding to the inhibitory receptor mFcγRIIb at a higher affinity than mFcγRIII (1,2).

Current methods used to measure ADCC rely on the isolation of primary peripheral blood mononuclear cells (PBMCs). 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 antibody drug screening and vaccine development programs.

The mFcγRIV ADCC Reporter Bioassay, Complete Kit[a,b] (Cat.# M1201), 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 mFcγRIV (3,4). The assay consists of a genetically engineered Jurkat T cell line that expresses:

- Mouse FcγRIV receptor
- A luciferase reporter driven by an NFAT-response element (NFAT-RE)

The mFcγRIV Effector Cells are provided in thaw-and-use format, which includes cryopreserved cells that can be thawed, plated and used in an assay without the need for propagation.

When cocultured with a target disease cell and relevant antibody, the mFcγRIV Effector Cells bind the Fc domain of the antibody, resulting in mFcγRIV 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.

The assay combines (1) a simple, add-mix-read single-day workflow with (2) mFcγRIV 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 mFcγRIV ADCC 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.

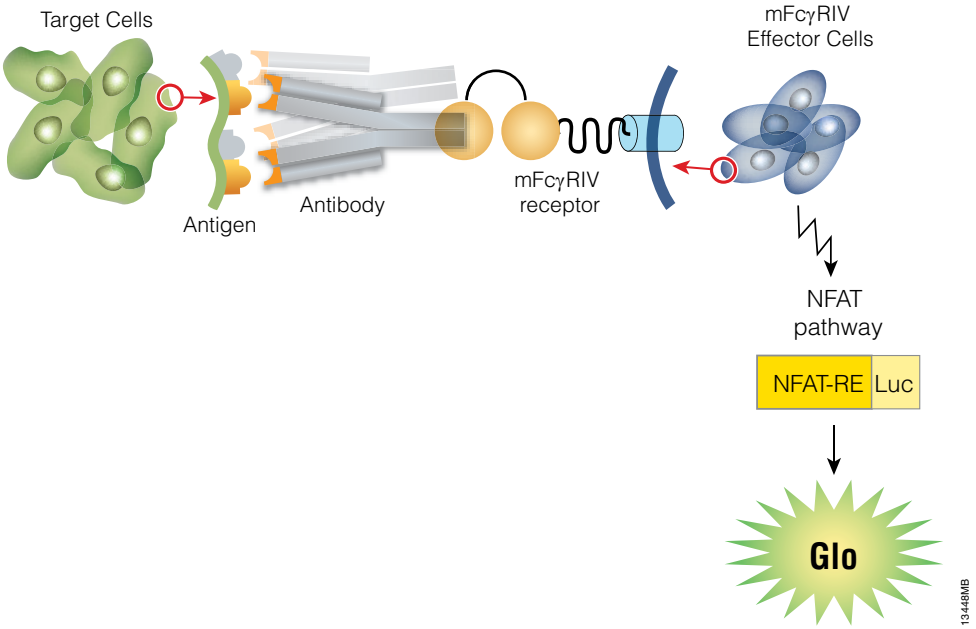


Figure 1. Representation of the mFcγRIV ADCC Reporter Bioassay. The bioassay consists of a genetically engineered cell line (mFcγRIV Effector Cells), an antigen expressing target cell and an antigen-specific antibody. When all components are cocultured, the antibody simultaneously binds the target cell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

The mFcγRIV ADCC Reporter Bioassay reflects the MOA of antibodies that bind and activate mFcγRIV. The bioassay shows high specificity as demonstrated using a mouse anti-HER2 IgG2b antibody and SKBR3 ($HER2^+$) cells, that together activate mFcγRIV Effector Cells but not FcγRIIIa Effector Cells (included in the human ADCC Reporter Bioassay, Cat. #G7015, G7014, G7010, G7018; Figure 2). Importantly, mFcγRIV-mediated luciferase activity is observed only in the presence of: 1) mFcγRIV Effector Cells; 2) an antibody with Fc binding affinity for mFcγRIV; and 3) target cells expressing the relevant antigen (Figure 3). Using a panel of anti-CD20 antibodies and Raji ($CD20^+$) target cells, the mFcγRIV ADCC Reporter Bioassay shows the expected rank ordering of human and mouse antibody subtypes (5; Figure 4).

1. Description (continued)

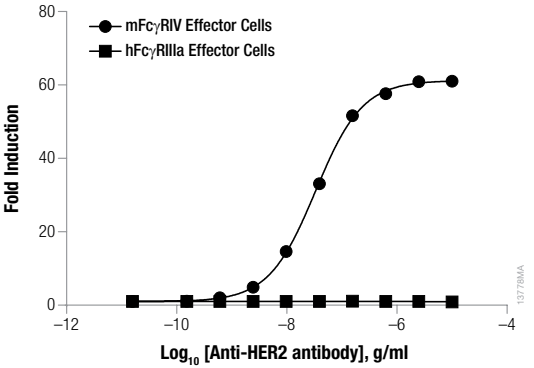


Figure 2. The mFcγRIV ADCC Reporter Bioassay reflects the MOA and specificity of antibodies designed to bind and activate mFcγRIV. Increasing concentrations of a mIgG2b anti-HER2 antibody were incubated with SKBR3 (HER2$^+$) target cells and either mFcγRIV Effector Cells or hFcγRIIIa Effector Cells (included in the human ADCC Reporter Bioassay, Cat. #G7015, G7014, G7010, G7018), as indicated. Bio-Glo™ Reagent was added, and luminescence was measured. Data were fitted to a 4PL curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

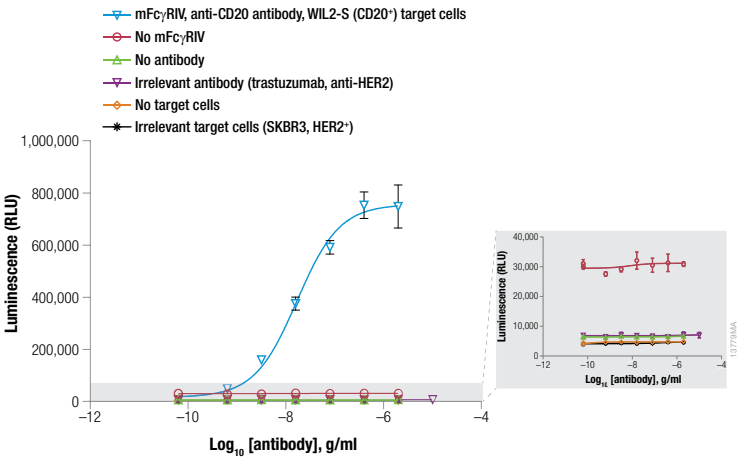


Figure 3. The mFcγRIV ADCC Reporter Bioassay is specific and requires mFcγRIV Effector Cells, an antibody with Fc binding affinity for mFcγRIV and target cells expressing the relevant antigen. Increasing concentrations of an anti-CD20 antibody (IgG2a) were incubated with WIL2-S (CD20$^+$) target cells and mFcγRIV Effector Cells. Bio-Glo™ Reagent was added, and luminescence was measured. Data were fitted to a 4PL curve using GraphPad Prism® software. No induction of luciferase activity was detected using effector cells that do not express mFcγRIV, in the absence of anti-CD20 antibody, or in the presence of an irrelevant antibody (trastuzumab, anti-HER2). No induction of luciferase activity was detected in the absence of target cells or in the presence of an irrelevant target cell (SKBR3, HER2$^+$).

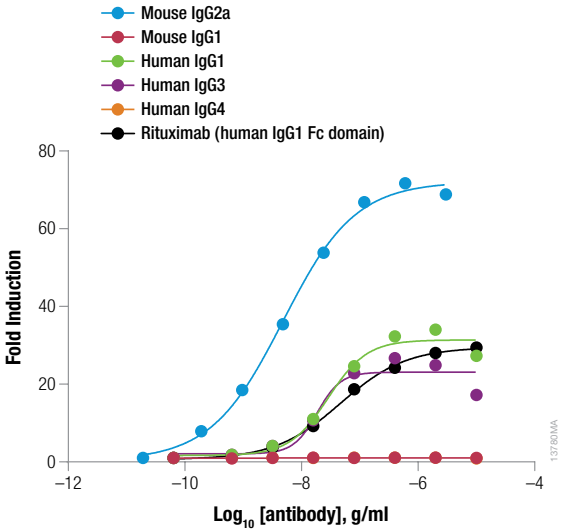


Figure 4. The mFcγRIV ADCC Reporter Bioassay shows the expected rank ordering of human and mouse antibody subtypes. Increasing concentrations of human and mouse anti-CD20 antibodies representing different IgG subtypes were incubated with Raji (CD20+) target cells and mFcγRIV Effector Cells, as indicated. Bio-Glo™ Reagent was added, and luminescence was measured. Data were fitted to a 4PL curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

Table 1. EC_{50} and Maximum Fold Induction for Human and Mouse Antibody Subtypes Analyzed in the mFcγRIV ADCC Reporter Bioassay.

Antibody Subtype	EC_{50} (ng/ml)	Maximum Fold Induction
mouse IgG2a	4.8	71.7
human IgG3	18.4	26.7
human IgG1	41.0	34.0
Rituximab (human IgG1 Fc domain)	46.7	29.4
human IgG4	NA	1.1
mouse IgG1	NA	1.0

NA = Not applicable.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
mFcγRIV ADCC Reporter Bioassay, Complete Kit	**1 each**	**M1201**

Not for Medical Diagnostic Use. Includes:

- 1 vial mFcγRIV Effector Cells (0.7ml)
- 1 vial Target Cells (Raji) (0.55ml)
- 36ml RPMI 1640 Medium
- 4ml Low IgG Serum
- 1 vial Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 10ml Bio-Glo™ Luciferase Assay Buffer

Note: The mFcγRIV ADCC Reporter Bioassay components are shipped separately because of temperature requirements. The mFcγRIV Effector Cells and Target Cells (Raji) 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.

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 Bio-Glo™ Luciferase Assay Substrate, Bio-Glo™ Luciferase Assay Buffer and Low IgG Serum at –30°C to –10°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. Store reconstituted Bio-Glo™ Luciferase Assay Reagent at –30°C to –10°C for up to 6 weeks.
- Store RPMI 1640 Medium at +2°C to +10°C protected from fluorescent light.

3. Before You Begin

The mFcγRIV ADCC Reporter Bioassay, Complete Kit, is intended to be used as a starter kit to gain familiarity with the mFcγRIV ADCC Reporter Bioassay, and for this purpose you should use the mFcγRIV Effector Cells and Target Cells (Raji) 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 mFcγRIV ADCC Reporter Bioassay, Core Kit (Cat.# M1211, M1215).

The mFcγRIV ADCC Reporter Bioassay differs from classic ADCC 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 ADCC assays using primary cells or other cell lines.

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

The mFcγRIV 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 mFcγRIV ADCC 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®-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® Cat.# 3370 or Linbro Cat.# 76-223-05)
- anti-CD20 control antibody (e.g., Beckman Coulter Cat.# A83477)
- white, flat-bottom 96-well assay plates (e.g., Corning® Cat.# 3917)
- sterile 15ml and 50ml conical tubes
- sterile reagent reservoirs (e.g., Corning® Cat.# 4870)
- 37°C, 5% CO_2 incubator
- 37°C water bath
- plate reader with glow luminescence measuring capability or luminometer (e.g., GloMax® Discover System)

4. Assay Protocol

This assay protocol illustrates the use of the mFcγRIV ADCC Reporter Bioassay to test two antibody samples against a control sample (Control Ab, Anti-CD20) in a single assay run. Each test and control 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 mFcγRIV ADCC 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 mFcγRIV Effector Cells (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. As a preliminary experiment, this can be simplified by using a single concentration of antibody (e.g., 2–5µg/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:** 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%.

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.

4. **Anti-CD20 Control Antibody, Sample:** Prepare a control anti-CD20 antibody according to the manufacturer's instructions. Place the reconstituted control antibody at 4°C until ready to use (Section 4.D).

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 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	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 an anti-CD20 control antibody 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 two 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 mFcγRIV ADCC Reporter Bioassay, Core Kit (Cat.# M1211, M1215).

1. Add 9.5ml 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 three 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 25µl of the cell suspension to each of the inner 60 wells of a 96-well, white, flat-bottom assay plate.
6. Add 75µl of assay buffer to each of the outside wells of the assay plates.
7. Cover the plates with lids and equilibrate the target cells for approximately 15 minutes at ambient temperature before adding antibody dilutions and mFcγRIV Effector Cells.

4.D. Preparing Antibody Serial Dilutions

The instructions described here are for preparation of a single stock of fivefold serial dilutions of a single antibody for analysis in triplicate (100µl of each dilution provides a sufficient volume for analysis in triplicate in two plates). Alternatively, you can prepare three independent stocks of serial dilutions to generate triplicate samples. To prepare fivefold serial dilutions, you will need 125µl of a control antibody at 3X the highest concentration for each dose-response curve (already prepared in Section 4.A). You will need 125µl 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.

1. Using a sterile clear 96-well plate, add 125µl of control Ab starting dilution (dilu1, 3X final concentration) to wells A11 and B11.
2. Add 125µl of test antibodies 1 and 2 starting dilution (dilu1, 3X final concentration) to wells C11 and D11, respectively (see Figure 6).
3. Add 100µl of assay buffer to other wells in these four rows, from column 10 to column 2.
4. Transfer 25µl of the antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent fivefold 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 120µl 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

1. 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.
2. Cover the assay plates with lids and keep the plates at ambient temperature before adding mFcγRIV Effector Cells.

4.F. Preparing and Plating mFcγRIV Effector Cells

Note: The thaw-and-use mFcγRIV 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.

1. Add 3.6ml of prewarmed (37°C) assay buffer to a 15ml conical tube.
2. Remove one vial of mFcγRIV Effector Cells 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 0.65ml 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 add 25µl of the cell suspension to each of the wells of the 96-well plates already containing target cells and antibody.
6. Cover the assay plates with a lid and incubate in a 37°C, 5% CO_2 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_{50} 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_{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).

5. 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 that the antibody is a subtype with affinity for the mFcγRIV receptor.
	Confirm, if known, the antibody affinity for the target cell antigen.
	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 mFcγRIV ADCC Reporter Bioassay will not necessarily be the same as determined from other ADCC 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 same 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 mFcγRIV Effector Cell density together with an increase in target cell density.
	Vary induction times within a range of 4–24 hours, and choose the induction time that gives the optimal response.
	If applicable, verify that the target cells still express antigen at the relevant passage number and method of harvesting.
	If applicable, 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.

5. Troubleshooting (continued)

Symptoms	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.
	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 mFcγRIV receptor or affect the NFAT-RE signaling pathway directly, causing a matrix effect. Use low-IgG serum or perform further dilution of antibody starting preparation to minimize this effect.
Will I see the same ranking of antibody potency in the Promega mFcγRIV ADCC Reporter Bioassay as in a classic ADCC bioassay?	The mFcγRIV ADCC Reporter Bioassay will measure antibody Fc-mediated signaling specifically through the mFcγRIV receptor, which data suggest is the primary Fc receptor through which antibodies mediate mouse ADCC in vivo (3–5). However, mFcγRI and mFcγRIII may also contribute to mouse ADCC function in vivo, and those receptors are not represented in the mFcγRIV ADCC Reporter Bioassay.

6. References

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3. Parekh, B.S. *et al.* (2012) Development and validation of an antibody-dependent cell-mediated cytotoxicity reporter gene assay. *mAbs* **4**, 310–8.
4. Surowy, T. *et al.* (2012) Low variability ADCC bioassay: Novel NFAT reporter bioassay for Fc effector function. *GEN* **32**, 28–9.
5. Overdijk, M.B. *et al.* (2012) Crosstalk between human IgG isotypes and murine effector cells. *J. Immunol.* **189**, 3430–8.

7. Appendix

7.A. Representative Assay Results

The following data were generated using the mFcγRIV ADCC Reporter Bioassay, Complete Kit including the mFcγRIV Effector Cells, Target Cells (Raji), and an anti-CD20 antibody, resulting in an EC_{50} value of 19.6ng/ml.

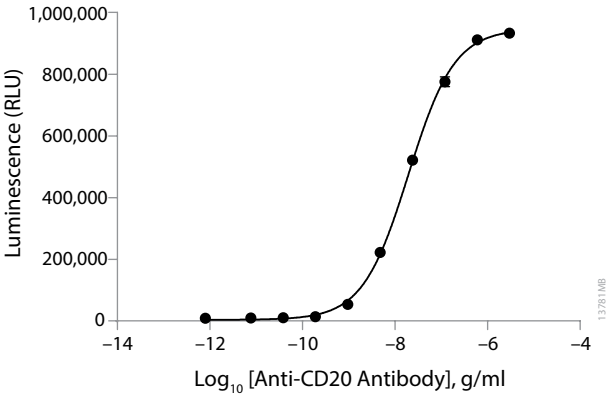


Figure 7. The mFcγRIV ADCC Reporter Bioassay reflects the MOA and specificity of an anti-CD20 antibody. Increasing concentrations of an anti-CD20 antibody were incubated with Target Cells (Raji) and mFcγRIV Effector Cells. Bio-Glo™ Reagent was added, and luminescence was measured. Data were fitted to a 4PL curve using GraphPad Prism® software.

8. Summary of Changes

The following changes were made to the 10/26 revision of this document:

1. Removed Section 7.B, Related Products.
2. Made minor text edits.

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