

TECHNICAL MANUAL

ADCP Reporter Bioassay (THP-1)

Instructions for Use of Products
JA9411 and JA9415

ADCP Reporter Bioassay (THP-1)

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1. Description

Antibody-dependent cellular phagocytosis (ADCP) is an important mechanism of action (MOA) of therapeutic antibodies designed to recognize and eliminate virus-infected or diseased (e.g., tumor) cells. Unlike antibody-dependent cellular cytotoxicity (ADCC), which is mediated primarily through FcγRIIIa expressed on natural killer (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 for measuring ADCP rely on isolating primary monocyte-derived macrophages and directly measuring phagocytosis. These assays are laborious and highly variable due to their reliance on donor cells, complex assay protocols and unqualified assay reagents. As a result, these assays are difficult to establish in quality-controlled, drug-development settings.

The ADCP Reporter Bioassay (THP-1)^(a-e) (Cat.# JA9411, JA9415) is a bioluminescent reporter cell-based assay that overcomes the limitations of existing assays. It can be used to measure the potency and stability of antibodies and other biologics with Fc domains that bind and activate FcγRI and/or FcγRIIa. The assay consists of a THP-1 monocyte cell line with endogenous expression of FcγRI and FcγRIIa (6,7). This cell line has been genetically engineered to express a NanoLuc[®] (NL) luciferase reporter driven by FcγR-dependent response elements.

The ADCP Reporter Bioassay Effector Cells (THP-1) are provided in thaw-and-use format as cryopreserved cells that can be thawed, plated and used in an assay without the need for cell culture and propagation. When cocultured with a target cell and relevant antibody, the ADCP Reporter Bioassay Effector Cells (THP-1) bind the Fc domain of the antibody, resulting in promoter-driven luminescence (Figure 1). The bioluminescent signal is quantified using the Bio-Glo-NL[™] Luciferase Assay System and a standard luminometer such as the GloMax[®] Discover System (Section 8.B, Related Products).

The ADCP Reporter Bioassay (THP-1) reflects the MOA of biologics designed to bind and activate FcγRI and/or FcγRIIa. FcγR-mediated luminescence is increased in a dose-dependent manner following the addition of Control Ab, Anti-CD20 (Cat.# GA1130) and Raji Target Cells (Cat.# G7016). Increased luminescence is dependent on the presence of an appropriate antibody and target cell (Figure 2).

The bioassay is prequalified following International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines and shows the precision, accuracy and linearity required for routine use in potency and stability studies (Table 1 and Figure 3). The bioassay can be performed in 1–2 days, and the workflow is simple, robust and compatible with both 96- and 384-well plate formats used for antibody screening in early drug discovery (Figure 4).

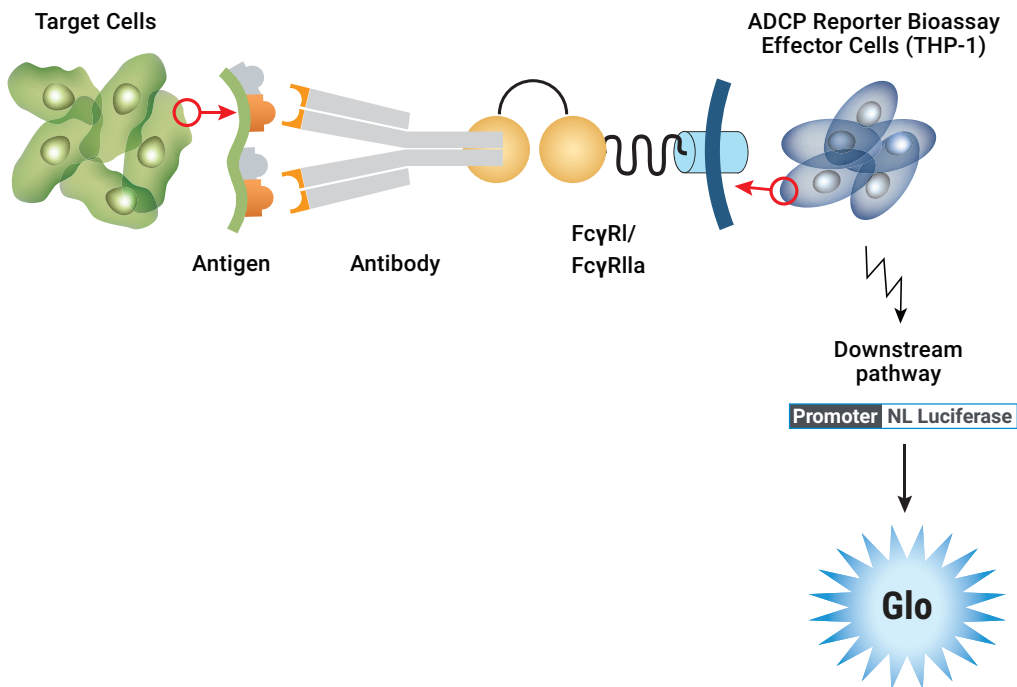


Figure 1. Representation of the ADCP Reporter Bioassay (THP-1). The bioassay consists of a genetically engineered cell line, ADCP Reporter Bioassay Effector Cells (THP-1), an antigen expressing target cell and an antigen-specific antibody. When all components are cocultured, the antibody simultaneously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

1. Description (continued)

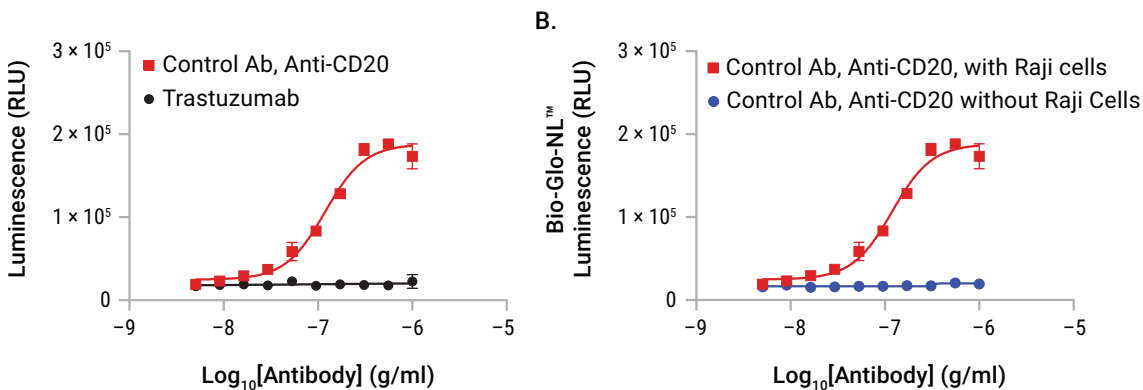


Figure 2. The ADCP Reporter Bioassay (THP-1) reflects the mechanism of action and specificity of antibodies designed to bind and activate FcγR. Panel A. ADCP Reporter Bioassay Effector Cells (THP-1) were incubated with serial dilutions of Control Ab, Anti-CD20 (Cat.# GA1130) or trastuzumab (Anti-HER2) in the presence of Raji target cells (CD20+/HER2-). **Panel B.** ADCP Reporter Bioassay Effector Cells (THP-1) were incubated with serial dilutions of Control Ab, Anti-CD20 in the presence or absence of Raji target cells. After a 4-hour incubation at 37°C, 5% CO₂, Bio-Glo-NL™ Reagent was added and luminescence was 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.

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Table 1. ADCP Reporter Bioassay (THP-1) Shows Precision, Accuracy and Linearity.

Parameter	Results	
	% Expected Relative Potency	% Recovery
Accuracy	50	100.9
	70	98.7
	100	101.4
	130	97.2
	150	97.9
Repeatability (% CV)	100% (Reference)	2.3
Intermediate Precision (% CV)		9.8
Linearity (r ²)		0.999
Linearity (y = mx + b)		y = 0.961x - 2.685

A 50–150% theoretical potency series of rituximab (Anti-CD20) was analyzed in triplicate in three independent experiments performed on three days by two analysts using the ADCP Reporter Bioassay (THP-1) with Raji target cells. Bio-Glo-NL™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were analyzed and relative potencies calculated after parallelism determination using JMP® software. Data were generated using thaw-and-use cells.

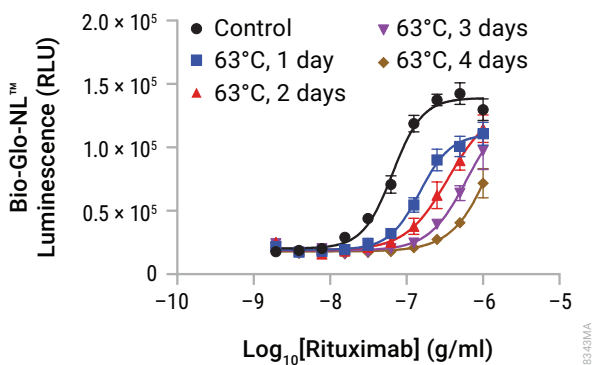


Figure 3. The ADCP Reporter Bioassay (THP-1) is stability-indicating. Samples of rituximab (Anti-CD20) were maintained at 4°C (control) or heat-treated at 63°C for the indicated times and analyzed using the ADCP Reporter Bioassay (THP-1) with Raji target cells. After a 4-hour incubation at 37°C, 5% CO₂, Bio-Glo-NL™ 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.

1. Description (continued)

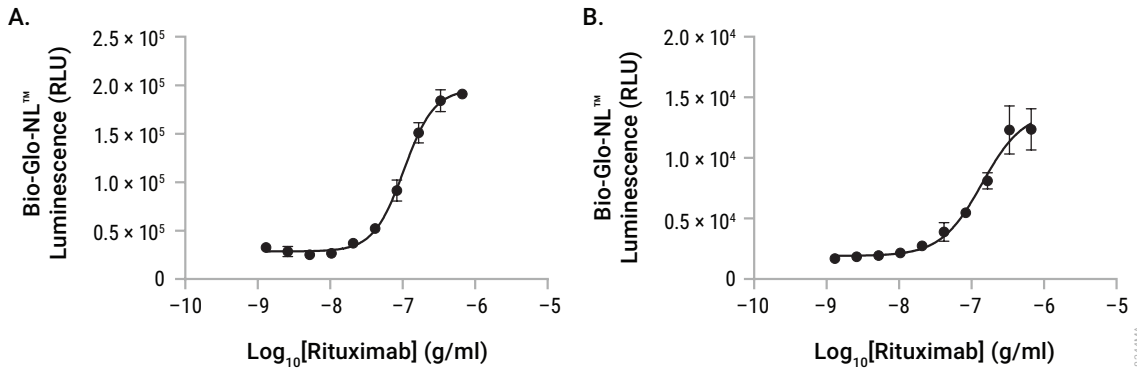


Figure 4. The ADCP Reporter Bioassay (THP-1) is amenable to 384-well plate format. Panel A. The ADCP Reporter Bioassay (THP-1) was performed in 96-well plates as described in this technical manual with a titration of rituximab (Anti-CD20) and Raji target cells. **Panel B.** The ADCP Reporter Bioassay (THP-1) was performed in 384-well white assay plates (e.g., Corning® Cat. # 3570) using: i) 8.3µl/well of 3X serially diluted rituximab, ii) 8.3×10^3 /8.3µl/well of Raji target cells; and iii) 1×10^4 /8.3µl/well ADCP Reporter Bioassay Effector Cells (THP-1). After a 4-hour incubation at 37°C, 5% CO₂, 25µl of Bio-Glo-NL™ Reagent was added per well and luminescence was quantified using the GloMax® Discover System. Data were fitted to four-parameter logistic curves using GraphPad Prism® software. The EC₅₀ values were 0.10 and 0.14µg/ml for the 96- and 384-well formats, respectively, and the fold induction was 6.0 and 8.2 for 96- and 384-well formats, respectively. Data were generated using thaw-and-use cells.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ADCP Reporter Bioassay (THP-1)	1 each	JA9411

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

- 1 vial ADCP Reporter Bioassay Effector Cells (THP-1) (0.5ml per vial)
- 36ml RPMI 1640 Medium
- 4ml Low IgG Serum
- 1 vial Bio-Glo-NL™ Luciferase Assay Substrate
- 10ml Bio-Glo-NL™ Luciferase Assay Buffer

PRODUCT	SIZE	CAT. #
ADCP Reporter Bioassay (THP-1), 5X	1 each	JA9415

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

- 5 vials ADCP Reporter Bioassay Effector Cells (THP-1) (0.5ml per vial)
- 5 × 36ml RPMI 1640 Medium
- 5 × 4ml Low IgG Serum
- 5 vials Bio-Glo-NL™ Luciferase Assay Substrate
- 5 × 10ml Bio-Glo-NL™ Luciferase Assay Buffer

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 decrease cell viability and cell performance.

Store Bio-Glo-NL™ Luciferase Assay Substrate, Bio-Glo-NL™ Luciferase Assay Buffer and Fetal Bovine Serum (FBS) at -30°C to -10°C . Avoid multiple freeze-thaw cycles of the serum. The Bio-Glo-NL™ Luciferase Assay Substrate remains liquid and does not freeze.


Store RPMI 1640 Medium at $+2^{\circ}\text{C}$ to $+10^{\circ}\text{C}$, protected from fluorescent light.

3. Before You Begin

The ADCP Reporter Bioassay (THP-1) differs from classic ADCP assays in a number of ways. Assay parameters including effector-to-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.

Remove the product label from the box containing vials with cells or note the catalog number, lot number and dispensed lot number from the label. This information can be used to download documents for the specified product from the web site, such as the Certificate of Analysis.

 **Note:** The ADCP Reporter Bioassay (THP-1) uses the Bio-Glo-NL™ Luciferase Assay System (Cat.# J3081, J3082, J3083) for detection. **Do not** use the Bio-Glo™ Luciferase Assay System (Cat.# G7940, G7941).

The ADCP Reporter Bioassay (THP-1) is intended to be used with user-provided antibodies and antigen-expressing target cells. The ADCC Reporter Bioassay, Target Kit (Raji) (Cat.# G7016) is available separately for use in routine quality control. The Target Kit contains Thaw-and-Use CD20+ Raji cells as well as Control Ab, Anti-CD20. We strongly recommend including the ADCC Reporter Bioassay, Target Kit (Raji) as a positive control in the first few assays to gain familiarity with the assay. Data generated using the ADCC Reporter Bioassay, Target Kit (Raji) are shown in Section 8, Representative Assay Results.

3. Before You Begin (continued)

The ADCP Reporter Bioassay Effector Cells (THP-1) are provided in frozen, thaw-and-use format and are ready to be used without any additional cell culture or propagation. When thawed, diluted and plated as instructed, the cells will be at 30,000 cells/well. The cells are sensitive, and care should be taken to follow cell thawing and plating procedures as described. Do not overmix or overwarm the cell reagents.

The ADCP Reporter Bioassay (THP-1) produces a bioluminescent signal and requires a sensitive luminescence plate reader. Bioassay development and performance data included in this Technical Manual were generated using the GloMax[®] Discover System (see Section 8.B, Related Products). An integration time of 0.5 second/well was used for all readings. The bioassay is compatible with most other plate-reading luminometers, though relative luminescence unit (RLU) readings will vary with the sensitivity and settings of each instrument. If using a reader with adjustable gain, we recommend a high-gain setting. The use of different instruments and gain adjustment will affect the magnitude of the raw data but should not affect the measured relative potency of test samples.

3.A. Materials to Be Supplied By the User

Reagents

- user-defined antibodies
- user-defined target cells
- sterile clear V-bottom 96-well 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; for best results use both manual and electronic pipettes as needed)
- sterile 15ml and 50ml conical tubes
- sterile reagent reservoirs (e.g., Corning[®]/Costar[®] Cat.# 4870)
- 37°C, 5% CO₂ incubator
- 37°C water bath
- sensitive plate reader with glow luminescence measuring capability or luminometer (e.g., GloMax[®] Discover System or equivalent system)
- **optional:** ADCC Reporter Bioassay, Target Kit (Raji); contains Raji Target Cells and Control Ab, Anti-CD20 (Cat.# G7016)

4. Preparing Cells, Reagents and Samples

This procedure illustrates the use of the ADCP Reporter Bioassay (THP-1) 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 ADCP Reporter Bioassay (THP-1) with your antibody and target cell line, we recommend optimizing the E:T (effector:target cell ratio) and cell densities. When thawed and plated as described in this Technical Manual, the ADCP Reporter Bioassay Effector Cells (THP-1) will be at 30,000 cells/well. We recommend keeping the effector cell number constant and varying the number of target cells (3,000–30,000 cells/well for a 96-well plate). For assay optimization, we recommend E:T in the range of 1:1 to 10:1. As a reference, we use Raji target cells and Control Ab, Anti-CD20 (Cat. # GA1130) at a ratio of 1.2:1, with 30,000 effector cells and 25,000 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 from 3–24 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:


- a. When using adherent target cells in the ADCP Reporter Bioassay (THP-1), target cells are plated the day before the assay. When suspension (nonadherent) target cells are used, the target cells are plated the day of the assay.
- b. For reference, we use 1 µg/ml of Control Ab, Anti-CD20 (Cat. # GA1130) as a starting concentration (1X) and twofold serial dilution.

4.A. Preparing Assay Buffer, Bio-Glo-NL™ Reagent and Antibody Samples

Assay Buffer: On the day of the assay, prepare 20ml of assay buffer (97% RPMI 1640/3% Low IgG Serum). Add 0.6ml of Low IgG Serum to 19.4ml RPMI 1640 Medium. Mix well and warm to 37°C before use. Warm the remaining RPMI 1640 Medium to 37°C.

Note: The recommended assay buffer contains 3% Low IgG Serum. This concentration of serum works well for most antibodies and target cells that we have tested. If you experience assay performance issues when using this assay buffer, we recommend testing different serum concentrations, in the range of 0.5–10%.

Bio-Glo-NL™ Luciferase Reagent: For reference, 10ml of Bio-Glo-NL™ Reagent is sufficient to assay 120 wells in a 96-well assay format. The Bio-Glo-NL™ Luciferase Assay Substrate should always be stored at –30°C to –10°C. Thaw the Bio-Glo-NL™ Luciferase Assay Buffer at room temperature (do not exceed 25°C) during the 4-hour assay induction period. We recommend preparing the reconstituted Bio-Glo-NL™ Luciferase Assay Reagent immediately before use. For instructions on use of the Bio-Glo-NL™ Luciferase Assay System, please refer to the *Bio-Glo-NL™ Luciferase Assay System Quick Protocol*, #FB227.

 **Note:** The ADCP Reporter Bioassay (THP-1) is compatible only with Bio-Glo-NL™ Luciferase Assay Reagent. **Do not use** Bio-Glo™ Luciferase Assay Reagent with the ADCP Reporter Bioassay (THP-1).

Test and Reference Samples: Using assay buffer as the diluent, prepare stock starting dilutions (dilu1, 3X final concentration) of two test antibodies (200µl each) and one reference antibody (400µl) in 1.5ml tubes. 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 between 0.25–1µg/ml (1X). Starting concentrations within this range have worked well for antibodies we have tested.
- b. For reference, if you are using the ADCC Reporter Bioassay, Target Kit (Raji), for testing the assay, prepare 400µl of a starting dilution of 3µg/ml (3X) of Control Ab, Anti-CD20 by adding 2.4µl of Control Ab, Anti-CD20 to 397.6µl of assay buffer.

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 antibody to generate two ten-point dose-response curves for 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 5. Example plate layout showing nonclustered sample locations of test antibody and reference antibody dilution series and wells containing assay buffer only (denoted by "B").

4.C. Preparing and Plating Target Cells

Target cells for use in the ADCP Reporter Bioassay (THP-1) 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 passage stability window. Both adherent and suspension target cells have been used successfully with the ADCP Reporter Bioassay (THP-1). Here we provide protocols for preparing both types of cells. Choose the one protocol that best suits your target cells.

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

Note: Perform the following steps in a sterile cell culture hood.

Preparing Adherent Target Cell Lines from Continuous Culture

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

1. Eighteen to twenty-four hours before the assay, aspirate the cell culture medium from the adherent target cells and wash with DPBS.
2. Add 2ml of Accutase® solution to each T75 flask and place the flask in a 37°C, 5% CO₂ incubator for 5–7 minutes or until the cells round up and detach from the bottom of the flask.
3. Add 8ml of antibiotic-free medium to the flask. Transfer the cell suspension to a 50ml (or larger) conical centrifuge tube.
4. Gently mix and count the adherent target cells by Trypan blue staining.
5. Centrifuge at 200 × *g* for 5 minutes.
6. Gently resuspend the cell pellet in antibiotic-free medium to achieve a concentration of 0.3–3 × 10⁵ viable cells/ml (3,000–30,000 cells per 100µl) as determined in optimization experiments for your target cells.
7. Transfer the suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 100µl of the cell suspension to the inner 60 wells of 96-well white flat-bottom assay plates.
8. Add 100µl of antibiotic-free medium to each of the outside wells of the assay plates.
9. Place lids on the assay plates and incubate in a 37°C, 5% CO₂ incubator overnight (18–24 hours).

4.C. Plating Target Cells (continued)

Preparing Suspension Target Cell Lines from Continuous Culture

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

1. On the day of the assay, prepare an appropriate amount of assay buffer as described in Section 4.A. Warm to 37°C.
2. Estimate the quantity of target cells needed, including extra volume to account for reservoir excess.
3. Gently mix and count the target cells by Trypan blue staining.
4. Harvest the required number of cells by centrifuging in a conical tube at 150 × *g* for 10 minutes.
5. Gently resuspend the pellet in assay buffer at 70% of the full volume needed to generate the targeted final cell density of 0.12–1.2 × 10⁶ viable cells/ml (3,000–30,000 cells per 25µl).
6. Count the cells again and adjust the volume of assay buffer to achieve the desired final cell density.
7. Transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 25µl of the cell suspension to the inner 60 wells of 96-well white flat-bottom assay plates.
8. Add 75µl of assay buffer to each of the outside wells of the assay plates.
9. Place lids on the assay plates and incubate at 37°C, 5% CO₂ while preparing antibody dilution series (Section 4.D).

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

1. On the day of the assay, prepare an appropriate amount of assay buffer as described in Section 4.A. Warm to 37°C.
2. Add 75µl of assay buffer to each of the outside wells, labeled “B” in Figure 5, of two white 96-well assay plates. Incubate plates at 37°C, 5% CO₂ to pre-equilibrate (10–15 minutes).
3. Label a 15ml conical tube “Target Cells (Raji)”. Add 4.5ml of assay buffer (prewarmed to 37°C) to the tube.
4. Remove one vial of ADCC Reporter Bioassay, Target Kit (Raji) cells from –140°C freezer storage or vapor phase of liquid nitrogen, immediately before use. Place on dry ice for transport to the bench. Thaw vial in a 37°C water bath until cells are just thawed (about 2–3 minutes). While thawing, gently agitate and visually inspect. Do not invert.



Note: This thawing protocol is recommended and important to the performance of the cells. No further handling is required or recommended.

5. Gently mix the cell suspension by pipetting 1–2 times. Transfer 0.5ml of cells to the tube labeled “Target Cells (Raji)” containing 4.5ml of assay buffer. Mix well by gently inverting the tube two times.
6. Transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 25µl of the cell suspension to the inner 60 wells of the assay plates, as defined in Figure 5.
7. Place lids on the assay plates and incubate at 37°C, 5% CO₂ while preparing the antibody dilution series (Section 4.D).

4.D. Preparing Antibody Serial Dilutions

These instructions are for preparation of a single stock of twofold serial dilutions of a single antibody for analysis in triplicate (100µl 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 twofold serial dilutions, you will need 400µl of reference antibody at 3X the highest antibody concentration in your dose-response curve. You will need 200µl 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.

Note: If you are using Control Ab, Anti-CD20 (Cat.# GA1130) as a control in the assay, follow the instructions below to prepare twofold serial dilutions. A twofold serial dilution for test antibodies is listed as an example below as well.

1. To a sterile clear V-bottom 96-well plate, add 200µl of reference antibody starting dilution (dilu1, 3X final concentration) to wells A11 and B11 (Figure 6).
2. Add 200µl of test antibodies 1 and 2 starting dilution (dilu1, 3X final concentration) to wells E11 and G11, respectively (Figure 6).
3. Add 100µl of assay buffer to other wells in these four rows, from column 10 to column 2.
4. Transfer 100µl of the antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent twofold 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 100µl 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 ADCP Reporter Bioassay Effector Cells (THP-1).

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		Reference Ab
B		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Reference Ab
C													
D													
E		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Test Ab 1
F													
G		no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Test Ab 2
H													

Figure 6. Example plate layout showing antibody serial dilutions.

4.E. Preparing ADCP Reporter Bioassay Effector Cells (THP-1)

1. Label a 15ml conical tube, "ADCP Effector Cells (THP-1)". Add 4.5ml of assay buffer (prewarmed to 37°C) to the tube.
2. On the day of the assay, remove one vial of ADCP Reporter Bioassay Effector Cells (THP-1) from -140°C freezer storage or vapor phase of liquid nitrogen, immediately before use. Place on dry ice for transport to the bench. Thaw vial in a 37°C water bath until cells are just thawed (about 2-3 minutes). While thawing, gently agitate and visually inspect. Do not invert.



Note: This thawing protocol is recommended and important to the performance of the cells. No further handling is required or recommended.

3. Gently mix the cell suspension by pipetting 1-2 times. Transfer 0.5ml of cells to the tube labeled "ADCP Effector Cells (THP-1)" containing 4.5ml of assay buffer. Mix well by gently inverting the tube two times.

5. Assay Protocol

5.A. Adding Antibodies and ADCP Reporter Bioassay Effector Cells (THP-1) to Adherent Target Cells

1. Remove the 96-well assay plate(s) containing preplated adherent target cells from 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, immediately add 25µl of assay buffer to the inner 60 wells of the assay plates.
3. Using a multichannel pipette, add 25µl of the appropriate antibody dilution (prepared in Section 4.D) to the plated target cells, according to the plate layout in Figure 5.
4. Transfer the ADCP Reporter Bioassay Effector Cells (THP-1) (prepared in Section 4.E) to a sterile reagent reservoir.
5. Using a multichannel pipette, immediately dispense 25µl of the effector cells to each of the inner 60 wells of the assay plates. Gently swirl the plates to ensure mixing of the target cells, effector cells and antibody.
6. Cover the assay plates with lids and incubate at 37°C, 5% CO₂ for 4 hours.

5.B. Adding Antibodies and ADCP Reporter Bioassay Effector Cells (THP-1) to Suspension Target Cells

1. Using a multichannel pipette, add 25µl of the appropriate antibody dilution (prepared in Section 4.D) to the assay plate(s) containing suspension target cells (Section 4.C), according to the plate layout in Figure 5.
2. Transfer the ADCP Reporter Bioassay Effector Cells (THP-1; prepared in Section 4.E) to a sterile reagent reservoir.
3. Using a multichannel pipette, immediately dispense 25µl of the effector cells to each of the inner 60 wells of the assay plates. Gently swirl the plates to ensure mixing of the target cells, effector cells and antibody.
4. Cover the assay plates with lids and incubate at 37°C, 5% CO₂ for 4 hours.

5.C. Preparing and Adding Bio-Glo-NL™ Reagent

We recommend preparing the Bio-Glo-NL™ Luciferase Assay Reagent immediately before use. Ensure that the Bio-Glo-NL™ Luciferase Assay Buffer is equilibrated to room temperature (do not exceed 25°C) before reconstituting the reagent. Do not store the reconstituted reagent. Once reconstituted, the reagent will lose 10% activity in approximately 8 hours at room temperature.



Note: The ADCP Reporter Bioassay (THP-1) is compatible only with Bio-Glo-NL™ Luciferase Assay Reagent. **Do not** use Bio-Glo™ Luciferase Assay Reagent with the ADCP Reporter Bioassay (THP-1).

1. Remove the Bio-Glo-NL™ Luciferase Assay Substrate from –30°C to –10°C storage and mix by pipetting. Briefly centrifuge the tubes if the substrate has collected in the cap or on the sides of the tubes.
2. Prepare the desired amount of reconstituted Bio-Glo-NL™ Luciferase Assay Reagent by combining one volume of substrate with 50 volumes of buffer. For example, if the experiment requires 10ml of reagent, add 200µl of substrate to 10ml of buffer. Ten milliliters (10ml) of Bio-Glo-NL™ Reagent is sufficient for 120 wells (two assay plates, using the inner 60 wells of each plate).

3. Remove assay plates from the incubator after the incubation period and equilibrate to room temperature for 10–15 minutes.
4. Using a manual multichannel pipette, add 75µl of Bio-Glo-NL™ Reagent to the inner 60 wells of the assay plates, taking care not to create bubbles.
5. Add 75µl of Bio-Glo-NL™ Luciferase Assay Reagent to wells B1, D1 and F1 of each assay plate to measure background signal.
6. Wait 5–10 minutes, then measure the luminescence in a GloMax® Discover System or a plate reader with glow-type luminescence reading capabilities. The luminescence intensity will decay gradually, with a signal half-life of approximately 120 minutes at room temperature.

Note: Varying the Bio-Glo-NL™ incubation time will affect the raw relative light unit (RLU) values but should not significantly change the EC₅₀ value and maximum fold induction.

5.D. Data Analysis

1. Determine the plate background by calculating the average RLU from wells B1, D1 and F1.
2. Calculate fold induction:

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

3. Graph data as RLU versus Log₁₀[antibody] and fold induction versus Log₁₀[antibody]. Fit curves and determine the EC₅₀ value 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. E-mail: techserv@promega.com

Symptoms	Causes and Comments
Low luminescence measurements (RLU readout)	<p>Ensure that you are using Bio-Glo-NL™ Reagent, which is designed for NanoLuc® Luciferase reporter bioassays. The ADCP Reporter Bioassay (THP-1) is not compatible with Bio-Glo™ Reagent, which is designed for firefly luciferase reporter bioassays.</p> <p>Choose an instrument designed for plate-reading luminescence detection. Instruments designed primarily for fluorescence detection are not recommended. Luminometers measure and report luminescence as relative values, and actual RLU numbers will vary between instruments.</p> <p>Some low-sensitivity luminometers models should be avoided. If using a reader with an adjustable gain, we recommend a high-gain setting.</p> <p>Insufficient cells per well can lead to low RLU. Handle and plate cells according to the instructions to ensure a sufficient number of viable cells per well.</p> <p>Low activity of Bio-Glo-NL™ Reagent leads to low RLU. Store and handle Bio-Glo-NL™ Reagent according to the instructions. For best results, prepare immediately before use.</p>
Weak assay response (low fold induction)	<p>Confirm, if known, the antibody's affinity for FcγRI and FcγRIIa.</p> <p>Optimize the concentration range of your test sample(s) to achieve a full dose response with complete upper and lower asymptotes. The EC₅₀ value obtained with the ADCP Reporter Bioassay (THP-1) may vary from the EC₅₀ value obtained using other methods such as primary macrophage-based assays.</p> <p>Increase the target cell density while maintaining the effector cell density. Since the readout of the assay is derived from the effector cells, improvement of the response can be achieved by increasing the number of target cells per well.</p> <p>Increase the ADCP Reporter Bioassay Effector Cells (THP-1) density together with an increase in target cell density.</p> <p>Optimize the assay incubation time within a range of 3–24 hours.</p> <p>Verify that the target cells still express antigen at the relevant passage number and method of harvesting.</p>

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Weak assay response (low fold induction) (continued)	<p>Verify that the target cells remain viable and ensure that you are following the recommended pre-assay culture conditions.</p> <p>The assay is sensitive to the concentration of FBS in assay buffer. Optimize the FBS concentration from 0.5–10% in assay buffer if assay performance is not ideal.</p> <p>If untreated control RLU is less than 100-fold above plate reader background RLU, subtract plate background RLU from all samples before calculating fold induction.</p>
Variability in assay performance.	<p>Variations in cell growth conditions including cell plating, harvest density, cell viability and cell doubling time can cause low assay performance and high assay variation. Avoid one-day cell passages whenever possible. Use high-quality cell culture reagents (especially serum) and plasticware for maintaining target cells in culture.</p> <p>Inappropriate cell counting methods can lead to variation in cell numbers in culture and assays and cause high assay variation. Ensure consistent and accurate cell counting methods.</p>

7. References

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8. Appendix

8.A. Representative Assay Results

The following data were generated using the ADCP Reporter Bioassay (THP-1) with with the ADCC Reporter Bioassay, Target Kit (Raji) (Figure 7).

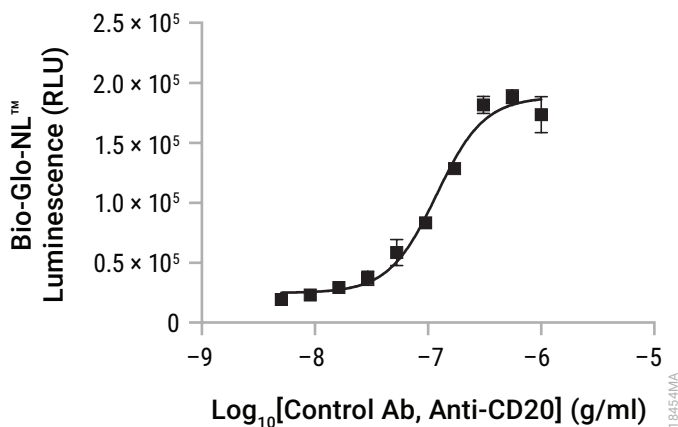


Figure 7. The ADCP Reporter Bioassay (THP-1) with Raji Target Cells measures the activity of Control Ab, Anti-CD20.

On the day of assay, Raji target cells, ADCP Reporter Bioassay Effector Cells (THP-1) and a titration of Control Ab, Anti-CD20 were added as described in this technical manual. After a 4-hour incubation at 37°C, 5% CO₂, Bio-Glo-NL™ Reagent was added and luminescence measured using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. The EC₅₀ value was 0.12µg/ml and fold induction was 9.8. Data were generated using thaw-and-use cells.

8.B. Related Products

Fc Effector Bioassays

Product	Size	Cat.#
ADCC Reporter Bioassay, Complete Kit (Raji)*	1 each	G7015
ADCC Reporter Bioassay, Core Kit*	1 each	G7010
ADCC Reporter Bioassay, F Variant, Core Kit**	1 each	G9790
ADCC Reporter Bioassay, Target Kit (Raji)*	1 each	G7016
FcγRIIIa-H ADCP Reporter Bioassay, Complete Kit**	1 each	G9901
FcγRIIIa-H ADCP Reporter Bioassay, Core Kit**	1 each	G9991
Mouse FcγRIV ADCC Bioassay, Complete Kit	1 each	M1201
Mouse FcγRIV ADCC Bioassay, Core Kit	1 each	M1211
Membrane TNFα Target Cells**	1 each	J3331
Membrane RANKL Target Cells**	1 each	J3381

*For Research Use Only. Not for use in diagnostic procedures.

**Not for Medical Diagnostic Use.

Additional kit formats are available.

Fc Effector Immunoassay

Product	Size	Cat.#
Lumit™ FcRn Binding Immunoassay	100 assays	W1151

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

Immune Checkpoint Bioassays

Product	Size	Cat.#
4-1BB Bioassay	1 each	JA2351
CD28 Bioassay	1 each	JA6701
CD28 Blockade Bioassay	1 each	JA6101
CD40 Bioassay	1 each	JA2151
CTLA-4 Blockade Bioassay	1 each	JA3001
GITR Bioassay	1 each	JA2291
ICOS Bioassay	1 each	JA6801
ICOS Blockade Bioassay	1 each	JA6001
LAG-3/MHCII Blockade Bioassay	1 each	JA1111
OX40 Bioassay	1 each	JA2191

Immune Checkpoint Bioassays (continued)

Product	Size	Cat. #
PD-1/PD-L1 Blockade Bioassay	1 each	J1250
PD-1+TIGIT Combination Bioassay	1 each	J2211
PD-L1 Negative Cells	1 each	J1191
TIGIT/CD155 Blockade Bioassay	1 each	J2201

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

Macrophage-Directed Bioassays

Product	Size	Cat. #
SIRP α /CD47 Blockade Bioassay	1 each	JA6011
SIRP α /CD47 Blockade Bioassay, Fc-Dependent	1 each	JA4801
TLR Bioassay	1 each	JA9011

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

T Cell Activation Bioassays

Product	Size	Cat. #
T Cell Activation Bioassay (IL-2)	1 each	J1651
T Cell Activation Bioassay (NFAT)	1 each	J1621
T Cell Activation Bioassay (TCR $\alpha\beta$ -KO, CD4+)	1 each	GA1172
T Cell Activation Bioassay (TCR $\alpha\beta$ -KO, CD8+)	1 each	GA1162
T Cell Activation Bioassay (TCR $\alpha\beta$ -KO, CD4+, CD8+)	1 each	GA1182

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

Cytokine and Growth Factor Bioassays

Product	Size	Cat. #
IL-2 Bioassay	1 each	JA2201
IL-6 Bioassay	1 each	JA2501
IL-12 Bioassay	1 each	JA2601
IL-15 Bioassay	1 each	JA2011
IL-23 Bioassay	1 each	JA2511
RANKL Bioassay	1 each	JA2701
VEGF Bioassay	1 each	GA2001

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

8.B. Related Products (continued)

Control Antibodies and Proteins

Product	Size	Cat.#
Control Ab, Anti-4-1BB	50µg	K1161
Control Ab, Anti-CD20	5µg	GA1130
Control Ab, Anti-CD40	50µg	K1181
Control Ab, Anti-CTLA-4	100µg	JA1020
Control Ab, Anti-LAG-3	100µg	K1150
Control Ab, Anti-OX40	50µg	K1191
Control Ab, Anti-PD-1	100µg	J1201
Control Ab, Anti-SIRPα	50µg	K1251
Control Ab, Anti-TIGIT	100µg	J2051
Control Ab, Anti-TIM-3	100µg	K1210
Recombinant VEGF ligand	10µg	J2371

Detection Reagents

Product	Size	Cat.#
Bio-Glo™ Luciferase Assay System	10ml	G7941
	100ml	G7940
Bio-Glo-NL™ Luciferase Assay System	10ml	J3081
	100ml	J3082
	1,000ml	J3083

Not for Medical Diagnostic Use.

Luminometers

Product	Size	Cat.#
GloMax® Navigator System	1 each	GM2000
GloMax® Discover System	1 each	GM3000
GloMax® Explorer System	1 each	GM3500

For Research Use Only. Not for use in diagnostic procedures.

Note: Additional Fc Effector, Immune Checkpoint, T Cell Activation and Cytokine Bioassays are available. To view and order Promega Bioassay products visit: www.promega.com/products/reporter-bioassays/ or visit Promega Tailored Solutions web site: www.promega.com/custom-solutions/tailored-solutions/ or e-mail: tailoredsolutions@promega.com

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