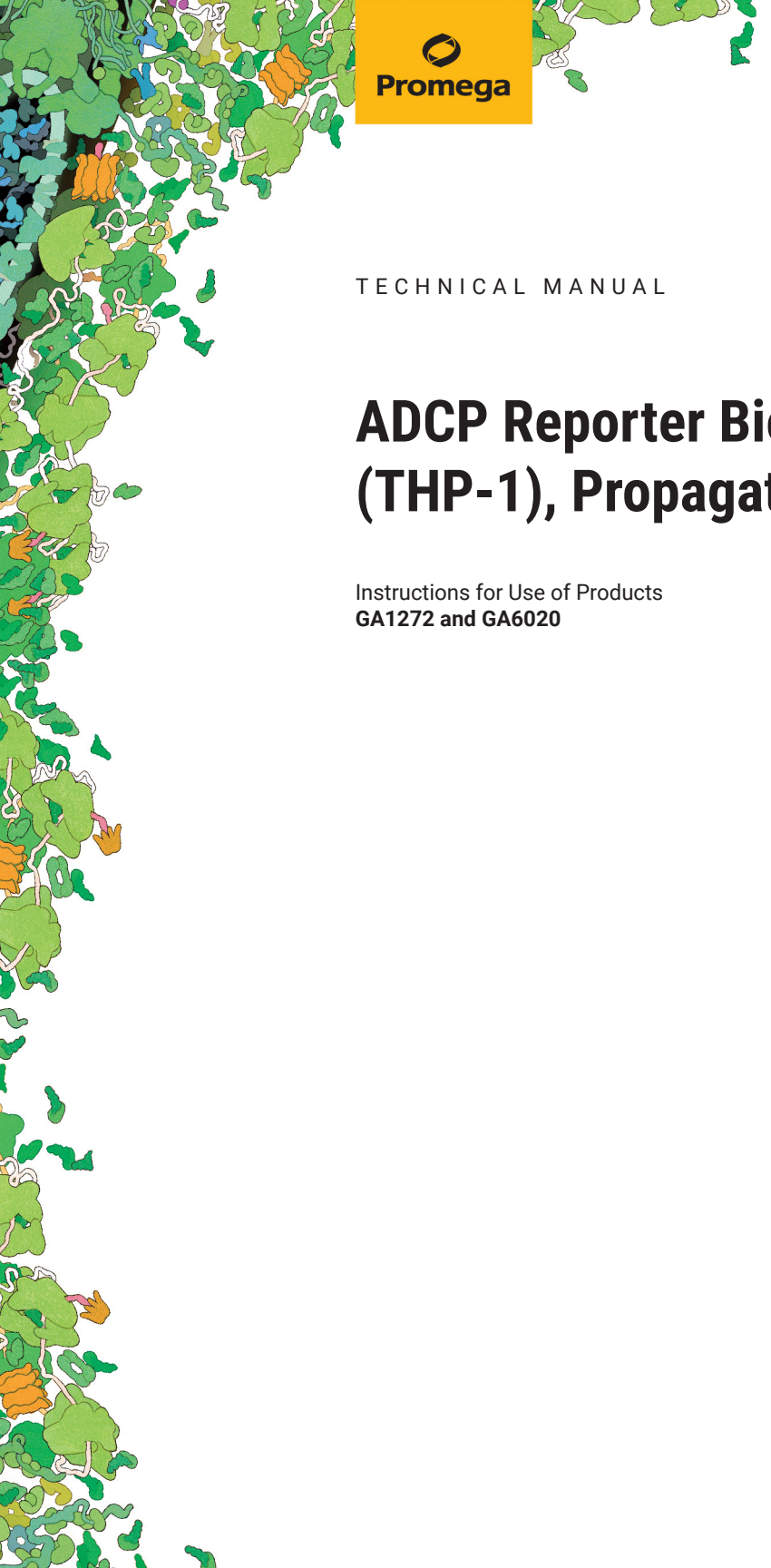

TECHNICAL MANUAL

ADCP Reporter Bioassay (THP-1), Propagation Model

Instructions for Use of Products
GA1272 and GA6020

Revised 4/25
TM700

Promega

ADCP Reporter Bioassay (THP-1), Propagation Model

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 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 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 direct measurement of 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), Propagation Model[a–d] (Cat.# GA1272) 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 Cell Propagation Model (CPM) format, which includes cryopreserved cells that can be thawed, propagated and banked for long-term use. Cell banks for the ADCP Reporter Bioassay Effector Cells (THP-1)[a–c] (Cat.# GA6020) are also available.

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 Harmonisation 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).

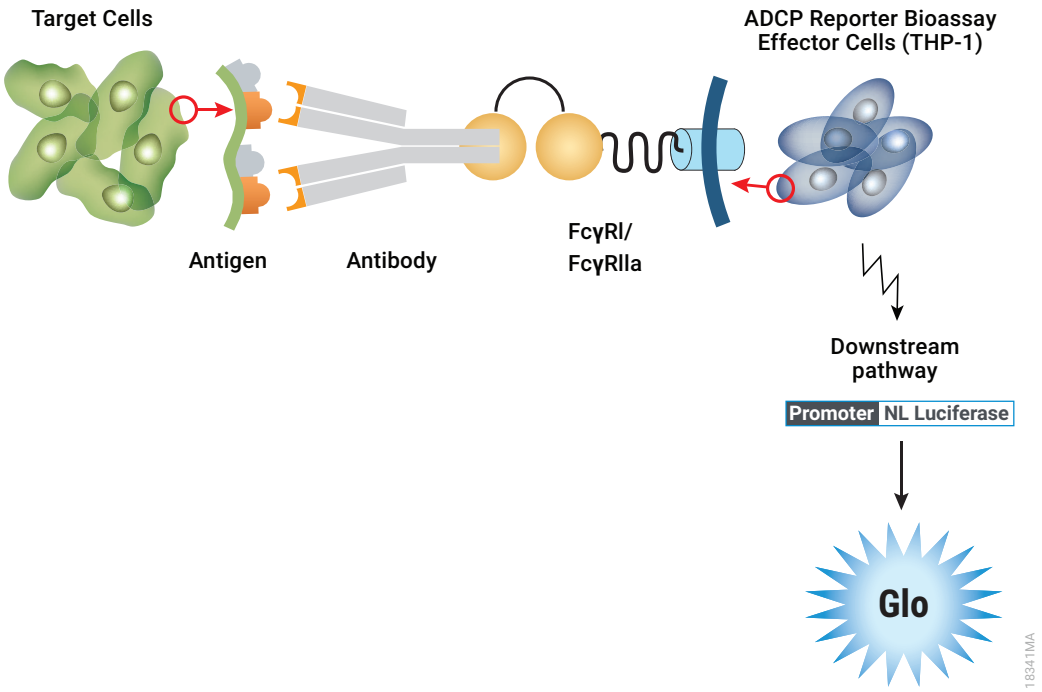

Figure 1. Representation of the ADCP Reporter Bioassay (THP-1). The bioassay consists of a genetically engineered cell line, ADCP Reporter Bioassay Effector Cells (THP-1), an antigen expressing target cell and an antigen-specific antibody. When all components are cocultured, the antibody simultaneously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

1. Description (continued)

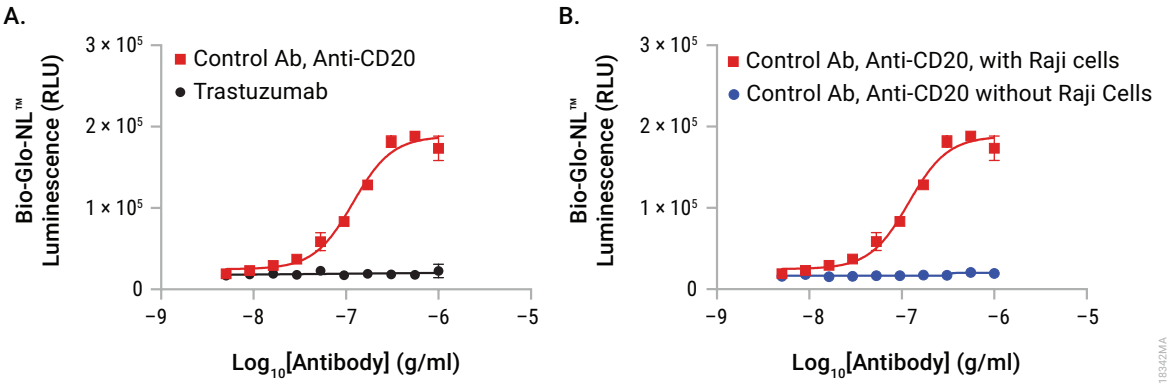

Figure 2. The ADCP Reporter Bioassay (THP-1) reflects the MOA and specificity of antibodies designed to bind and activate FcγR. Panel A. ADCP Reporter Bioassay Effector Cells (THP-1) were incubated with serial dilutions of Control Ab, Anti-CD20 or trastuzumab (Anti-HER2) in the presence of Raji target 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% CO_2, 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.

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

Parameter	Results	
Accuracy	% Expected Relative Potency	% Recovery
	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.

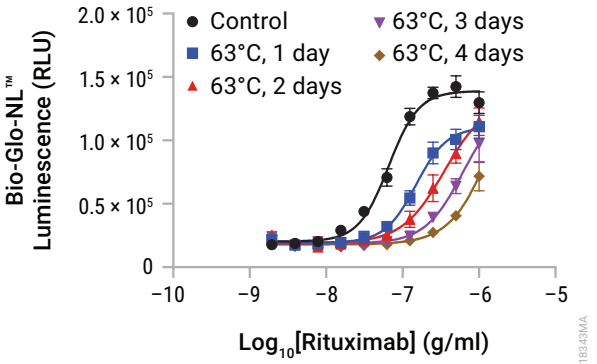

Figure 3. The ADCP Reporter Bioassay (THP-1) is stability-indicating. Samples of rituximab (Anti-CD20) were maintained at 4°C (control) or heat-treated at 63°C for the indicated times and analyzed using the ADCP Reporter Bioassay (THP-1) with Raji target cells. After a 4-hour incubation at 37°C, 5% CO_2, 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)

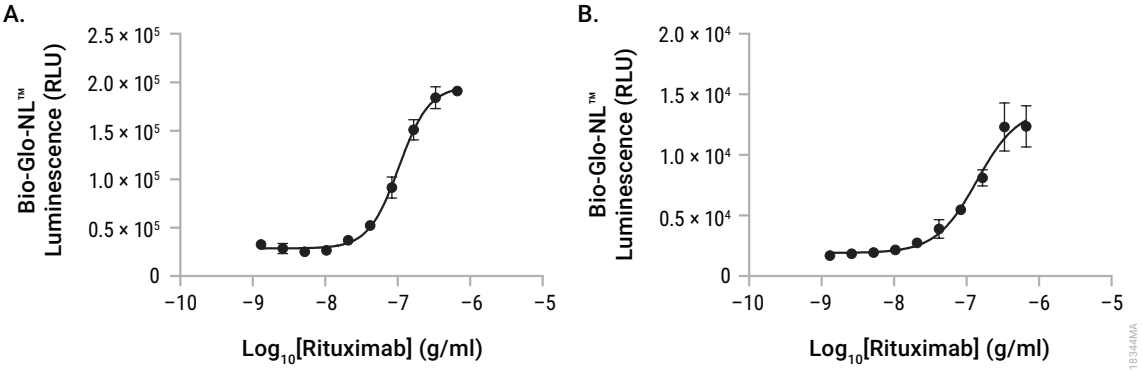

Figure 4. The ADCP Reporter Bioassay (THP-1) is amenable to 384-well plate format. Panel A. The ADCP Reporter Bioassay (THP-1) was performed in 96-well plates as described in this technical manual with a titration of rituximab (Anti-CD20) and Raji target cells. **Panel B.** The ADCP Reporter Bioassay (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% CO_2, 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 EC_{50} 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), Propagation Model	**1 each**	**GA1272**

Not for Medical Diagnostic Use. Includes:

- 2 vials ADCP Reporter Bioassay Effector Cells (THP-1) (CPM), 1.32 × 10^7 cells/ml (1.0ml per vial)

PRODUCT	SIZE	CAT.#
ADCP Reporter Bioassay Effector Cells (THP-1), Cell Bank	**1 each**	**GA6020**

Not for Medical Diagnostic Use. Includes:

- 50 vials ADCP Reporter Bioassay Effector Cells (THP-1) (CPM), 1.32 × 10^7 cells/ml (1.0ml per vial)

Note: Thaw and propagate one vial to create frozen cell banks before use in an assay. The remaining vial(s) should be reserved for future use.

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.

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.

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

Note: The ADCP Reporter Bioassay (THP-1), Propagation Model, 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).

Cell thawing, propagation and banking should be performed exactly as described in Section 4. Cell seeding and propagation densities have been optimized to ensure stable cell growth, which is reflected in a steady cell doubling rate to achieve optimal and consistent performance. By contrast, the recommended cell plating densities, induction time and assay buffer components described in Section 5 were established using a small panel of antigen-expressing target cells and antibodies that activate FcγR. You may need to adjust these parameters and optimize assay conditions for your own antibodies or other biologic samples.

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.

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3.A. Materials to Be Supplied by the User

Reagents

- user-defined antibodies
- user-defined target cells
- RPMI 1640 medium with L-glutamine and HEPES (e.g., Corning® Cat.# 10-041-CV or GIBCO® Cat.# 22400105)
- fetal bovine serum (FBS; e.g., VWR Cat.# 89510-194, GIBCO® Cat.# 35-015-CV or HyClone Cat.# SH30071.03)
- fetal bovine serum, ultra-low IgG (Thermo Fisher Cat.# 16250)
- hygromycin B (e.g., GIBCO® Cat.# 10687010)
- DMSO (e.g., Sigma Cat.# D2650)
- Accutase® solution (e.g., Sigma Cat.# A6964)
- DPBS (e.g., GIBCO® Cat.# 14190144)
- Bio-Glo-NL™ Luciferase Assay System (Cat.# J3081, J3082, J3083)
- Trypan blue solution (e.g., Sigma Cat.# T8154)
- **optional:** Control Ab, Anti-CD20 (Cat.# GA1130)
- **optional:** ADCC Reporter Bioassay, Target Kit (Raji) (Cat.# G7016)

Supplies and Equipment

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

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

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

Table 2. Quick Guide to Media Compositions.

	Cell Thawing Medium	Cell Growth Medium	Cell Freezing Medium
RPMI 1640	90%	90%	85%
FBS	10%	10%	10%
Hygromycin	–	200µg/ml	–
DMSO	–	–	5%

4.A. Cell Thawing and Initial Cell Culture

1. Prepare 20ml of cell thawing medium (Table 2) by adding 2ml of FBS to 18ml of RPMI 1640 medium prewarmed to 37°C. Transfer 9ml of prewarmed cell thawing medium to a 15ml conical tube.
2. Remove one vial of ADCP Reporter Bioassay Effector Cells (THP-1) from storage at –140°C and thaw in a 37°C water bath with gentle agitation (do not invert cell vial) until just thawed (typically 2–3 minutes).
3. Transfer all of the cells (approximately 1ml) to the 15ml conical tube containing 9ml of prewarmed cell thawing medium.
4. Centrifuge at 150 × *g* for 10 minutes.
5. Carefully aspirate the medium and resuspend the cell pellet in 7ml of prewarmed cell thawing medium.
6. Transfer the cell suspension to a T25 tissue culture flask and place the flask horizontally in a humidified 37°C, 5% CO_2 incubator.
7. Incubate for approximately 24 hours before passaging the cells.
8. Passage the cells at a seeding density of 5×10^5 viable cells/ml using cell growth medium containing antibiotics (Table 2).

   Note: When passaging cells for the first time after thawing, it is critical to use a minimum seeding density of 5×10^5 viable cells/ml. Lower densities may reduce cell viability and growth.
9. Incubate for approximately 48 hours before passaging the cells according to the schedule outlined in Section 4.B.

4.B. Cell Maintenance and Propagation

For cell maintenance and propagation, use cell growth medium containing antibiotics and monitor cell viability and doubling rate during propagation. The cell growth rate will stabilize by 7–10 days after thawing, at which time cell viability is typically >90%, and the average cell doubling rate is 48 hours. Passage number should be recorded for each passage. In our experience, cells maintain their functionality for up to 25 passages, or 29 cell doublings, if passaging is performed on a Monday-Wednesday-Friday schedule.

1. On the day of cell passage, measure cell viability and density by Trypan blue staining.
2. Seed the cells at a density of 5.0×10^5 cells/ml if passaging every two days (e.g., Monday-Wednesday or Wednesday-Friday) or 3.0×10^5 cells/ml if passaging every three days (e.g., Friday-Monday). Always maintain the flasks in a horizontal position in the incubator. Do not allow the cells to grow to a density greater than 1×10^6 cells/ml.
3. Maintain the cell culture by adding fresh cell growth medium to the cell suspension in the original flask or by transferring the cells to a new flask while maintaining a consistent ratio of culture volume to flask surface area (e.g., 25ml volume per T75 flask or 50ml volume per T150 flask).
4. Place the flasks horizontally in a humidified, 37°C, 5% CO_2 incubator.

4.C. Cell Freezing and Banking

1. On the day of cell freezing, prepare fresh cell freezing medium (Table 2) and keep on ice.
2. Gently mix the cells with a pipette to create a homogenous cell suspension.
3. Remove a sample for cell counting by Trypan blue staining. Calculate the volume of cell freezing medium needed based on desired cell freezing densities of 1×10^7–2×10^7 cells/ml.
4. Transfer the cell suspension to 50ml sterile conical tubes or larger-sized centrifuge tubes and centrifuge at 150 × *g*, 4°C for 10–15 minutes.
5. Gently aspirate the medium, taking care not to disturb the cell pellet.
6. Carefully resuspend the cell pellet in ice-cold cell freezing medium to a final cell density of 1×10^7–2×10^7 cells/ml. Combine the cell suspensions into a single tube and dispense into cryovials.
7. Freeze the cells using a controlled-rate freezer (preferred), or a Mr. Frosty® or a Styrofoam® rack in a –80°C freezer overnight. Transfer the vials to –140°C or below for long-term storage.

5. Assay Protocol

This procedure illustrates the use of the ADCP Reporter Bioassay (THP-1), Propagation Model, to test two antibody samples against a reference sample in a single assay run. Each test and reference antibody is run in triplicate, in a ten-point dilution series, in a single 96-well assay plate using the inner 60 wells. Other experimental and plate layouts are possible but may require further optimization.

Prior to routine use of the ADCP Reporter Bioassay (THP-1), Propagation Model with your antibody and target cell line, we recommend optimizing the E:T (effector:target cell ratio) and cell densities. Fix the number of ADCP Reporter Bioassay Effector Cells (THP-1) at 30,000 cells/well for a 96-well plate and vary the number of target cells (3,000–30,000 cells/well for a 96-well plate). This will help to establish an E:T and cell density that give a strong signal response and fold induction. For assay optimization, try E:T in the range of 1:1 to 10:1. As a reference, with Raji target cells and Control Ab, Anti-CD20 (Cat. # GA1130), we use 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 in a range of 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:

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

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

Assay Buffer: On the day of the assay, prepare an appropriate amount of assay buffer (97% RPMI 1640/3% ultra-low IgG FBS). Mix well and warm to 37°C before use. For reference, 40ml of assay buffer is typically sufficient for 120 wells in a 96-well assay format using the inner 60 wells.

Note: The recommended assay buffer contains 3% ultra-low IgG FBS. This concentration of FBS 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 (150µl each) and one reference antibody (300µl) in 1.5ml tubes. Store the tubes containing antibody starting dilutions appropriately before making antibody serial dilutions.

Notes:

- 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.
- If you are using Raji target cells with Control Ab, Anti-CD20 for testing the assay, prepare 300µl of a starting dilution of 3µg/ml (3X) Control Ab, Anti-CD20 by adding 1.8µl of Control Ab, Anti-CD20 to 298.2µl of assay buffer.

5.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").

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

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).

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

1. Passage the adherent target cells two days before plating for the assay to ensure optimal and consistent assay performance.
2. Aspirate the cell culture medium from the adherent target cells and wash with DPBS.
3. Add 2ml of Accutase® solution to each T75 flask, and place the flask in a 37°C, 5% CO_2 incubator for 5–7 minutes or until the cells round up and detach from the bottom of the flask.
4. Add 8ml of antibiotic-free medium to the flask. Transfer the cell suspension to a 50ml (or larger) conical centrifuge tube.
5. Gently mix and count the adherent target cells by Trypan blue staining.
6. Centrifuge at 200 × *g* for 5 minutes.
7. Gently resuspend the cell pellet in antibiotic-free medium to achieve a concentration of 0.3–3 × 10^5 viable cells/ml (3,000–30,000 cells per 100µl) as determined in optimization experiments for your target cells.
8. Transfer the suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 100µl of the cell suspension to each of the inner 60 wells of a 96-well white flat-bottom assay plate.
9. Add 100µl of antibiotic-free medium to each of the outside wells of the assay plates.
10. Place lids on the assay plates and incubate in a 37°C, 5% CO_2 incubator overnight (18–24 hours).

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

Target cells for use in the 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 window of passage stability.

1. Passage the suspension target cells two days before plating for the assay to ensure optimal and consistent assay performance.
2. On the day of the assay, prepare an appropriate amount of assay buffer as described in Section 5.A. Warm to 37°C.
3. Estimate the quantity of target cells needed, including extra volume to account for reservoir excess.
4. Gently mix and count the target cells by Trypan blue staining.
5. Harvest the required number of cells by centrifuging in a conical tube at 150 × *g* for 10 minutes.
6. 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 × 10^6 viable cells/ml (3,000–30,000 cells per 25µl).

7. Count the cells again and adjust the volume of assay buffer to achieve the desired final cell density.
8. Incubate the target cell suspension at 37°C to maintain the temperature while preparing antibody dilution series.

5.E. Preparing Antibody Serial Dilutions

The instructions described here are for preparation of a single stock of threefold 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 threefold serial dilutions, you will need 300µl of reference antibody at 3X the highest antibody concentration in your dose-response curve. You will need 150µl 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 threefold serial dilutions. A threefold serial dilution for test antibodies is listed as an example below as well.

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.

5.E. Preparing Antibody Serial Dilutions (continued)

1. To a sterile clear V-bottom 96-well plate, add 150µl of reference antibody starting dilution (dilu1, 3X final concentration) to wells A11 and B11 (Figure 6).
2. Add 150µl 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 50µl of the antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent threefold 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).

5.F. Adding Antibodies to Preplated Adherent Target Cells

1. Remove the 96-well assay plates containing 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 to the plated target cells, according to the plate layout in Figure 5.
4. Cover the assay plates with lids and let sit at room temperature while preparing the effector cells.

5.G. Plating Suspension Target Cells and Antibodies

1. Gently mix the previously prepared target cell suspension (Section 5.D).
2. Add the target cell suspension to a reagent reservoir. Using a multichannel pipette, immediately dispense 25µl of the cells to each of the inner 60 wells of a 96-well flat-bottom, white, assay plate.
3. Using a multichannel pipette, add 25µl of the appropriate antibody dilution to the plated target cells, according to the plate layout in Figure 5.
4. Cover the assay plates with lids and let sit at room temperature while preparing the effector cells.

5.H. Preparing and Adding ADCP Reporter Bioassay Effector Cells (THP-1)

While maintaining the ADCP Reporter Bioassay Effector Cells (THP-1), it is important to follow the recommended cell seeding density. Changes in cell culture volume or seeding density will affect cell growth rate and assay performance. Only use the cells in the assay after the cell doubling rate has stabilized during propagation, and when cell viability is ≥90%.

1. Passage the cells two days before performing the assay as described in Section 4.B.
2. Count the ADCP Reporter Bioassay Effector Cells (THP-1) by Trypan blue staining and calculate the cell density and viability.
3. Transfer an appropriate amount of ADCP Reporter Bioassay Effector Cells (THP-1) from the culture vessel to a 50ml conical tube or larger sized centrifuge tube.
4. Pellet the cells at 150 × *g* for 10 minutes at ambient temperature, and resuspend the pellet in assay buffer at 70% of the full volume needed to generate the targeted final cell density of 1.2×10^6 cells/ml.
5. Count the cells again and adjust the volume of assay buffer to achieve a final cell density of 1.2×10^6 cells/ml (30,000 cells per 25µl). You will need at least 5ml of ADCP Reporter Bioassay Effector Cells (THP-1) to fill 120 assay wells or the inner 60 wells of two assay plates.
6. Mix the ADCP Reporter Bioassay Effector Cells (THP-1) by inverting the tube and transfer the suspension to a reagent reservoir.
7. Using a multichannel pipette, immediately dispense 25µl of the effector cells to each of the inner 60 wells of the assay plates containing target cells and antibodies. Gently swirl the assay plates to ensure mixing of the target cells, effector cells and antibody.
8. Add 75µl of assay buffer to each of the outside wells of the assay plates.
9. Cover the assay plates with lids and incubate at 37°C, 5% CO_2 for 3–24 hours, as determined in optimization experiments for your cells.

5.I. 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 EC_{50} value and maximum fold induction.

5.J. Data Analysis

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

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

3. Graph data as RLU versus Log_{10} [antibody] and fold induction versus Log_{10} [antibody]. Fit curves and determine the EC_{50} 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**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Low luminescence measurements (RLU readout)	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.
	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 values will vary between instruments.
	Some models of luminometers with low sensitivity should be avoided. If using a reader with an adjustable gain, we recommend a high-gain setting.
	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.
	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.
Weak assay response (low fold induction)	Confirm, if known, the antibody's affinity for FcγRI and FcγRIIa.
	Optimize the concentration range of your test sample(s) to achieve a full dose response with complete upper and lower asymptotes. The EC_{50} value obtained with the ADCP Reporter Bioassay (THP-1) may vary from the EC_{50} value obtained using other methods such as primary macrophage-based assays.
	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.
	Increase the ADCP Reporter Bioassay Effector Cells (THP-1) density together with an increase in target cell density.
	Optimize the assay incubation time within a range of 3–24 hours.
	Verify that the target cells still express antigen at the relevant passage number and method of harvesting.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Weak assay response (low fold induction) (continued)	Verify that the target cells remain viable and ensure that you are following the recommended pre-assay culture conditions.
	Verify the viability and density of the ADCP Reporter Bioassay Effector Cells (THP-1) at harvesting (Section 5.H).
	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.
	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.
Variability in assay performance.	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, especially when using ADCP Reporter Bioassay Effector Cells (THP-1). Use high-quality cell culture reagents (especially serum) and plasticware for maintaining cells in culture. Ensure consistent cell growth by handling the cells exactly according to the instructions.
	Inappropriate cell handling during cell harvest, including long centrifuge times and high centrifuge speeds can cause low assay performance and high assay variation. Centrifuge the cells exactly according to the instructions.
	Inappropriate cell freezing/DMSO exposure can cause low assay performance and high assay variation. Freeze the cells exactly according to the instructions.
	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.

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), Propagation Model, with Raji target cells and Control Ab, Anti-CD20 (Cat.# GA1130) or rituximab (Figure 7).

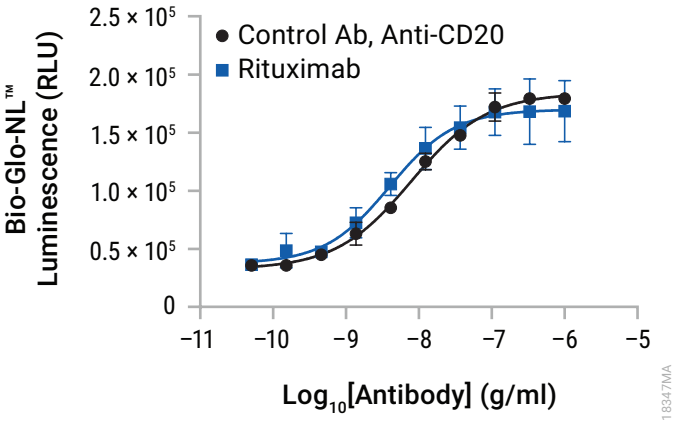

Figure 7. The ADCP Reporter Bioassay (THP-1) with Raji Target Cells measures the activity of anti-CD20 antibodies. On the day of assay, Raji target cells, ADCP Reporter Bioassay Effector Cells (THP-1) and a titration of Control Ab, Anti-CD20, or rituximab (Anti-CD20) were added as described in this technical manual. After a 4-hour incubation at 37°C, 5% CO_2, 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. EC_{50} values were 7.9 and 4.0ng/ml for Control Ab, Anti-CD20, and rituximab, respectively, and fold induction was 5.2 and 4.7 for Control Ab, Anti-CD20 and rituximab, respectively.

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
FcγRIIa-H ADCP Reporter Bioassay, Complete Kit**	1 each	G9901
FcγRIIa-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.

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8.B. Related Products (continued)

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
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.#
SIRPα/CD47 Blockade Bioassay	1 each	JA6011
SIRPα/CD47 Blockade Bioassay, Fc-Dependent	1 each	JA4801
TLR Bioassay	1 each	JA9011
ADCP Reporter Bioassay (THP-1)	1 each	JA9411

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 (TCRαβ-KO, CD4+)	1 each	GA1172
T Cell Activation Bioassay (TCRαβ-KO, CD8+)	1 each	GA1162
T Cell Activation Bioassay (TCRαβ-KO, CD4+, CD8+)	1 each	GA1182

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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.

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.

8.B. Related Products (continued)

Luminometers

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

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Note: Additional Fc Effector, Immune Checkpoint, T Cell Activation, Cytokine, Macrophage, Primary Cell and Target Cell Killing Bioassays are available. To view and order Promega Bioassay products visit: **www.promega.com/products/reporter-bioassays/** or email: **EarlyAccess@promega.com**.

For information on custom bioassay development and services visit the Promega Tailored R&D Solutions website: **www.promega.com/custom-solutions/tailored-solutions/**

9. Summary of Changes

The following changes were made to the 4/25 revision of this document:

1. Added a patent statement and removed an expired patent statement.
2. Corrected text defining ICH in Section 1, Description.
3. Updated the Lumit trademark and a third party trademark.
4. Applied patent statements to Cat.# GA6020.
5. Updated text in Section 3 regarding the cell vial box label.

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[b]U.S. Pat. Nos. 8,557,970, 8,669,103, 9,777,311, 9,840,730, 9,951,373, 10,233,485, 10,633,690, 10,774,364, 10,844,422, 11,365,436, 11,661,623, 11,667,950; European Pat. Nos. 2456864, 2635595, 2990478, 3181687, 3409764; Japanese Pat. Nos. 6038649, 6155424, 6227615, 6374420, 6539689; and other patents and patents pending.

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