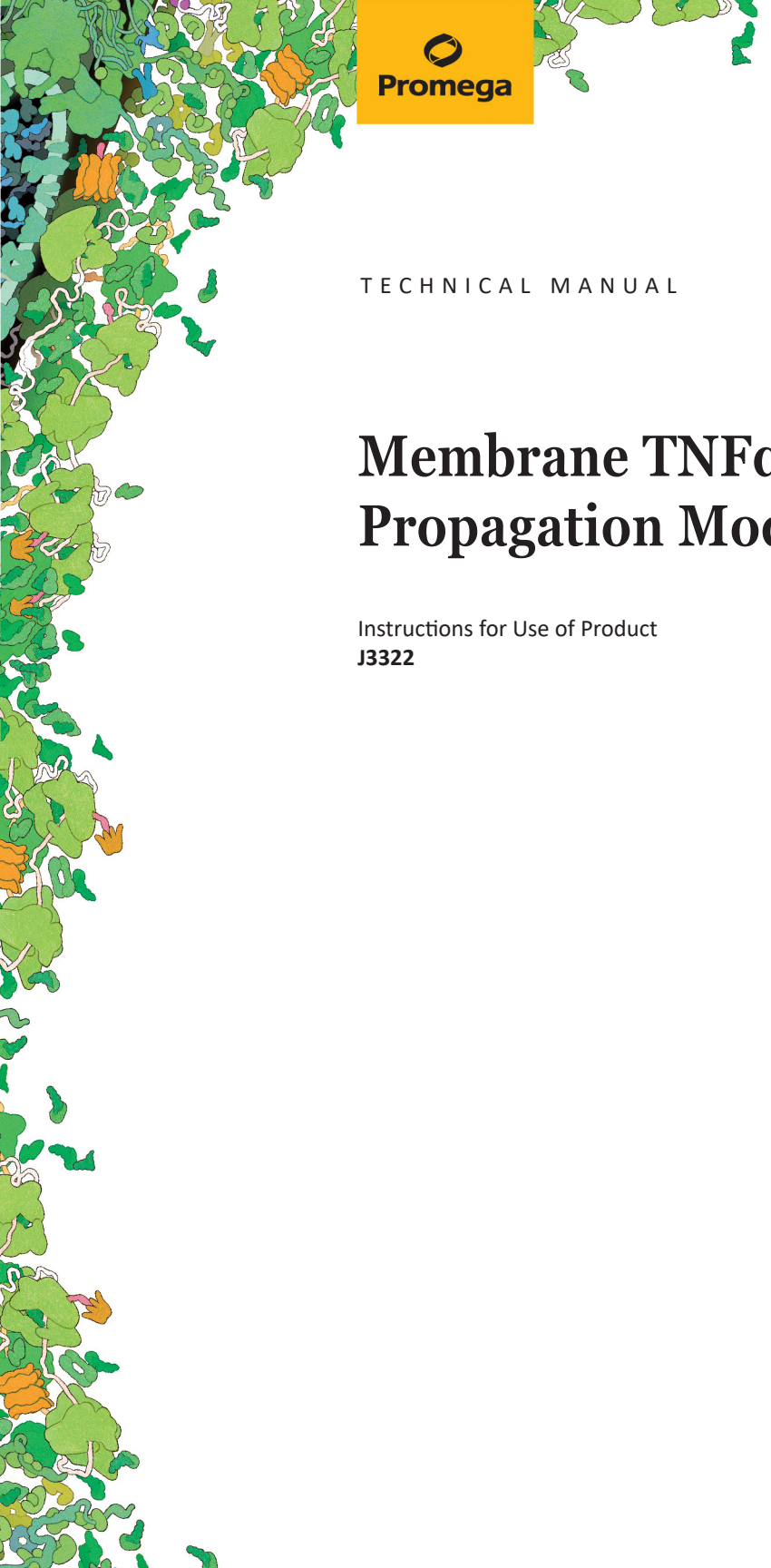

TECHNICAL MANUAL

Membrane TNFα Target Cells, Propagation Model

Instructions for Use of Product
J3322

11/21
TM664

Membrane TNFα Target Cells, Propagation Model

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

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1. Description

Tumor necrosis factor alpha (TNFα) is a key cytokine involved in immune and inflammatory responses. TNFα is produced primarily by macrophages but can be generated by other leukocytes as well as endothelial cells, cardiomyocytes and other cell types. TNFα binding to its receptors, TNF receptor type 1 (TNFR1) and TNFR2, induces a myriad of cellular responses that are cell-context dependent. Typically, TNFα binding to TNFR1 and/or TNFR2 induces pro-inflammatory responses, such as immune cell activation, promotion of effector functions and cytokine production, as well as the acute phase response and the hallmarks of inflammation: fever, swelling, redness and pain (1). In vivo, TNFα exists in both a trimeric membrane-bound form (mTNFα) and as a soluble protein. TNFα is produced as a type II membrane protein and cleaved by the metalloproteinase TNFα-converting enzyme (TACE, ADAM17; 2,3). Once cleaved, the soluble trimeric TNFα is biologically active, but a less potent activator of TNFR2 compared to mTNFα (4).

TNFα has a significant role in the pathology of several inflammatory and autoimmune disorders, including rheumatoid arthritis and ulcerative colitis (5,6). Blockade of TNFα/TNFR binding using neutralizing antibodies or engineered TNFR fusion proteins has proven to be a viable strategy for providing clinical benefit in inflammatory diseases. Furthermore, targeting TNFα-producing cells for destruction may provide additional advantages to reduce the inflammatory burden. Specifically, binding of TNFα-targeted antibodies to mTNFα-expressing cells can induce effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) to destroy the mTNFα-expressing inflammatory cells (7,8). Several FDA-approved TNFα antibodies have been confirmed to possess ADCC and CDC function. However, determining the ability of novel and biosimilar TNFα antibodies to induce cellular cytotoxicity is hampered by the lack of model cell lines naturally expressing mTNFα.

Membrane TNFα Target Cells, Propagation Model[a,b] (Cat.# J3322), is a genetically engineered cell line stably expressing a cleavage-resistant form of mTNFα that enforces its surface expression. mTNFα Target Cells are provided in cell propagation model (CPM) format, which includes cryopreserved cells that can be thawed, propagated and banked for long-term use. They are designed to be used as target cells in assays that measure the effector functions, such as ADCC and CDC, for anti-TNFα blockers. In addition, they can be used to measure antibody binding affinity to mTNFα.

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mTNFα Target Cells express TNFα on the cell surface, as demonstrated by flow cytometry (Figure 1). The assay signal is specific to anti-TNFα antibodies in both ADCC and CDC assays. Using the ADCC Reporter Bioassay (Cat.# G7010), luminescence increases after adding anti-TNFα antibodies, but not after adding anti-VEGF or anti-CD20 antibodies (Figure 2). In a CDC assay, mTNFα Target Cell death is detected after adding anti-TNFα antibodies, but not after adding anti-VEGF or anti-CD20 antibodies (Figure 3). The ADCC Reporter Bioassay using mTNFα Target Cells is prequalified following International Council for Homogenization 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 4). The bioassay can be performed in a two-day timeframe, 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 5).

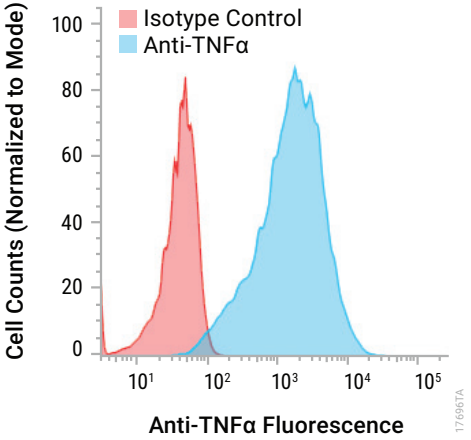

Figure 1. Surface expression of TNFα on mTNFα Target Cells. mTNFα Target Cells were labeled with isotype control or anti-TNFα (infliximab) followed by AlexaFluor® 488-conjugated anti-human IgG. Cells were analyzed on a BD LSRFortessa™ X-20 flow cytometer. Data analysis was performed with FlowJo™ software. Data were generated using thaw-and-use cells.

1. Description (continued)

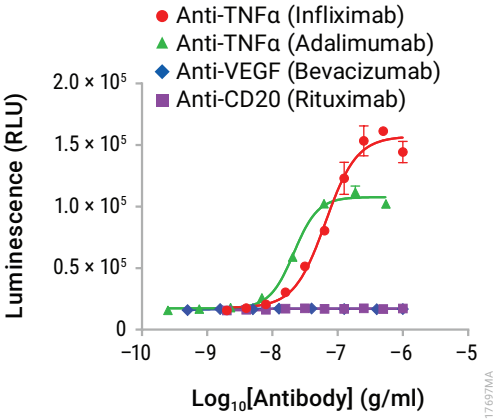

Figure 2. The ADCC Reporter Bioassay performed with mTNFα Target Cells reflects the mechanism of action (MOA) and shows specificity for antibodies designed to bind TNFα. ADCC Effector Cells were cocultured with mTNFα Target Cells in the presence of serial titrations of antibodies, as indicated. After a 6-hour induction, Bio-Glo™ Reagent was added, and luminescence quantified using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

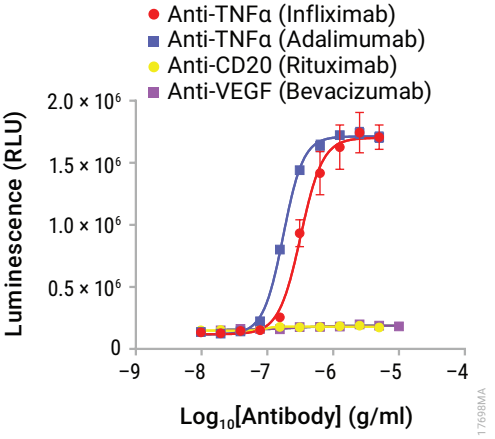

Figure 3. CDC assay with mTNFα Target Cells reflects the MOA and shows specificity for antibodies designed to bind TNFα. mTNFα Target Cells were incubated with 10% normal human serum complement in the presence of serial titrations of antibodies, as indicated. After a 6-hour incubation, CytoTox-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

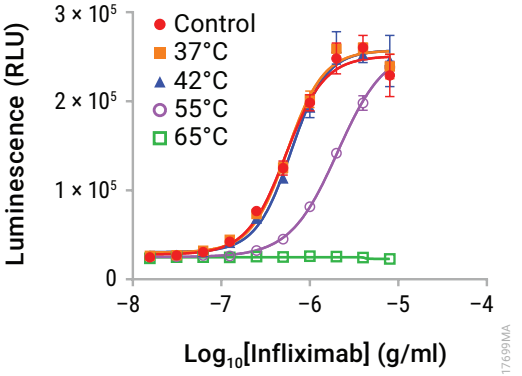

Figure 4. The ADCC Reporter Bioassay with mTNFα Target Cells is stability-indicating. Samples of anti-TNFα (infliximab) were maintained at 4°C (control) or heat-treated for 24 hours at the indicated temperatures, then analyzed using the ADCC Reporter Bioassay with mTNFα Target Cells. Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

Table 1. ADCC Reporter Bioassay using mTNFα Target Cells Shows Precision, Accuracy and Linearity.

Parameter	**Results**	
Accuracy	% Expected Relative Potency	% Recovery
	50	49.1
	70	72.0
	100	100.9
	140	150.1
	200	213.4
Repeatability (% CV)	100% (Reference)	2.1
Intermediate Precision (% CV)		9.4
Linearity (r^2)		0.9991
Linearity (y = mx + b)		y = 1.099x – 6.024
A 50–200% simulated potency series of infliximab was analyzed in triplicate in three independent experiments performed on three days by two analysts using mTNFα Target Cells in the ADCC Reporter Bioassay. Bio-Glo™ 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.		

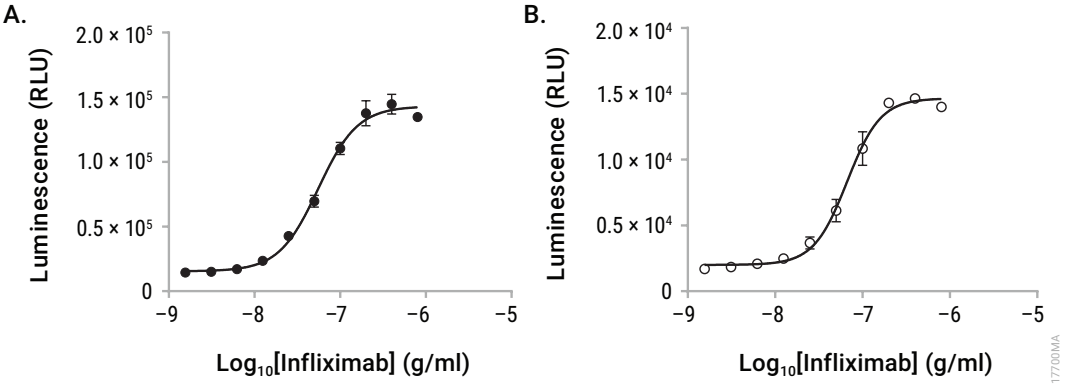

Figure 5. The ADCC Reporter Bioassay with mTNFα Target Cells is amenable to 384-well plate format. **Panel A.** The bioassay was performed in 96-well plates as described in this technical manual with a titration of anti-TNFα (infliximab). **Panel B.** The bioassay was performed with mTNFα Target Cells in 384-well plates, as described here. Thaw-and-use mTNFα Target Cells were thawed and plated at 2×10^3/15µl/well 20 hours prior to the assay, in a 384-well white assay plate. On the assay day, 5µl of a serial dilution (1:2) of 5X concentrated infliximab was added to the wells, followed by addition of 1.5×10^4/5µl/well ADCC Effector Cells. After a 6-hour induction at 37°C, 5% CO_2, 25µl/well of Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to four-parameter logistic curves using GraphPad Prism® software. The EC_{50} values were 55 and 67ng/ml for 96- and 384-well formats, respectively. The fold induction was 9.3 and 7.3 for 96- and 384-well formats, respectively. Data were generated using thaw-and-use cells.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Membrane TNFα Target Cells, Propagation Model	**1 each**	**J3322**

Not for Medical Diagnostic Use. Includes:

- 2 vials Membrane TNFα Target Cells (CPM), 4×10^6 cells/ml (1.0ml per vial)

Storage Conditions: Upon arrival, immediately transfer the cell vials to below –140°C (freezer or liquid nitrogen vapor phase) 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.

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

3. Before You Begin

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 and lot number from the label. This information can be used to download documents from the specified product from the website such as Certificate of Analysis.

Note: mTNFα Target Cells are intended to be used with user-provided antibodies or other biologics designed to bind to TNFα. Data generated using infliximab (Remicade®) and adalimumab (Humira®) are shown in Section 9.A, Representative Assay Results.

To measure ADCC activity, mTNFα Target Cells can be used in conjunction with the ADCC Reporter Bioassay, Propagation Model (Cat.# G7102) or ADCC Reporter Bioassay (thaw-and-use; Cat. #G7010, G7018) to detect ADCC function of anti-TNFα antibodies. To measure CDC activity, we recommend using CytoTox-Glo™ Reagent (Cat.# G9290) with normal human serum complement.

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, consistent performance. An accurate, reliable and reproducible cell counting method is required for routine cell culturing and optimal bioassay performance.

The recommended cell plating densities, induction time and assay buffer components described in Sections 5 and 6 were established using infliximab and ADCC Effector Cells (ADCC Reporter Bioassay) or in a CDC assay using CytoTox-Glo™ Reagent and complement-preserved human serum. You may need to adjust the parameters provided here and optimize assay conditions for your own assay readout and antibodies.

The ADCC Reporter Bioassay and the CDC assay with CytoTox-Glo™ Reagent produce a bioluminescent signal and require a sensitive luminescence plate reader. Bioassay development and performance data included in this Technical Manual were generated using the GloMax® Discover System (see Section 9.C, Related Products). An integration time of 0.5 second/well was used for all readings. These bioassays are 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. Before You Begin (continued)

Materials to Be Supplied by the User

(Composition of buffers and solutions is provided in Section 9.B.)

Reagents

- user-defined anti-TNFα antibodies or other biologics samples (e.g., infliximab NDC 57894-030-01)
- Ham's F-12 Medium with L-glutamine (e.g., GIBCO™ Cat.# 11765062)
- fetal bovine serum (FBS; e.g., GIBCO™ Cat.# 35-015-CV or HyClone Cat.# SH30071.03)
- G418 Geneticin (e.g., GIBCO™ Cat.# 10131)
- DMSO (e.g., Sigma Cat.# D2650)
- Accutase® solution (e.g., Sigma Cat.# A6964)
- DPBS (e.g., GIBCO™ Cat.# 14190)
- Trypan blue solution (e.g., Sigma Cat.# T8154)
- **optional:** ADCC Bioassay Effector Cells, Propagation Model (Cat.# G7102)
- **optional:** Super Low IgG FBS (e.g., HyClone Cat.# SH30898; for ADCC Bioassay)
- **optional:** Bio-Glo™ Luciferase Assay System (Cat.# G7940, G7941; for ADCC Bioassay)
- **optional:** RPMI 1640 with L-glutamine and HEPES (GIBCO™ Cat.# 22400; for ADCC Bioassay)
- **optional:** Normal Human Serum Complement (Quidel, Cat.#A113; for CDC assay)
- **optional:** CytoTox-Glo™ Cytotoxicity Assay (Cat.# G9290; for CDC assay)

Supplies and Equipment

- solid-white, flat-bottom 96-well assay plates (e.g., Corning® Cat.# 3917) for plating and reading luminescence
- sterile clear V-bottom 96-well plate with lid (e.g., Costar® Cat.# 3896) for preparing antibody dilutions
- sterile dilution reservoirs with lid (e.g., Dilux™ Cat.# D-1002) for higher volume 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., Costar®/Corning® 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; for ADCC Reporter Bioassay or CDC assay using CytoTox-Glo™ Assay)

4. Preparing mTNFα Target Cells

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

4.A. Cell Thawing and Initial Cell Culture

1. Prepare 50ml of initial cell culture medium by adding 5ml of FBS to 45ml of Ham's F12 medium prewarmed to 37°C. This initial cell culture medium will be used for culturing the cells immediately after thawing.
2. Transfer 9ml of prewarmed initial cell culture medium to a 50ml conical tube.
3. Remove one vial of mTNFα Target Cells from storage at −140°C and thaw in a 37°C water bath with gentle agitation (do not invert) until just thawed (typically 2–3 minutes).
4. Transfer all of the cells (approximately 1ml) to the 50ml conical tube containing 9ml of prewarmed initial cell culture medium.
5. Centrifuge at 150 × *g* for 10 minutes.
6. Carefully aspirate the medium and resuspend the cell pellet in 40ml of prewarmed initial cell culture medium.
7. Transfer the cell suspension to a T150 tissue culture flask and place the flask horizontally in a 37°C, 5% CO_2 incubator.
8. Incubate for approximately 48 hours before passaging the cells.

4.B. Cell Maintenance and Propagation

For cell maintenance and propagation starting from the second cell passage, use the cell growth medium containing antibiotics and monitor cell viability and doubling rate during propagation. The cell growth rate will stabilize by 7–10 days post-thaw, at which time cell viability is typically >95%, and the average cell doubling rate is ~24 hours. Passage number should be recorded for each passage. In our experience, cells maintain their functionality for up to 25 passages if passaging is performed on a Monday-Wednesday-Friday schedule.

1. On the day of cell passage, aspirate the cell culture medium and wash the cells with DPBS.
2. Add 2ml of Accutase® solution to each T75 flask (or 4ml of Accutase® solution for T150) and place 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.
3. Add 8ml of cell growth medium to each T75 flask (or 16ml of cell growth medium for T150 flask). Transfer the cell suspension to a sterile 15ml or 50ml conical tube.
4. Count the cells by Trypan blue staining. We suggest seeding the cells at a density of 4×10^4 cells/cm^2 if passaging every two days (e.g., Monday-Wednesday or Wednesday-Friday) or 2×10^4 cells/cm^2 if passaging every three days (e.g., Friday-Monday).
5. Add an appropriate amount of cell growth medium to a new flask.
6. Transfer the appropriate volume of cell suspension to achieve the desired cell seeding density per area.
7. 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, make fresh cell freezing medium and keep on ice.
2. Aspirate the cell culture medium and wash the cells with DPBS.
3. Add 2ml of Accutase® solution to each T75 flask and place 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 cell growth medium to each T75 flask. Transfer the cell suspension to a sterile 15ml or 50ml conical tube.
5. 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 3×10^6–1.2×10^7 cells/ml.
6. Transfer the cell suspension to 50ml sterile conical or larger-sized centrifuge tubes, and centrifuge at $150 \times g$, 4°C for 10 minutes.
7. Carefully aspirate the supernatant and avoid disturbing the cell pellet.
8. Gently resuspend the cell pellet in ice-cold cell freezing medium to a final cell density of 3×10^6–1.2×10^7 cells/ml. Combine the cell suspensions into a single tube and dispense into cryovials.
9. 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 at or below –140°C for long-term storage.

5. Assay Protocol for ADCC Reporter Bioassay using mTNFα Target Cells

This assay protocol requires two engineered cell lines: ADCC Bioassay Effector Cells, Propagation Model, and Membrane TNFα Target Cells, Propagation Model. Both cell lines are also available in thaw-and-use format (Cat.# G7010 and Cat.# J3331, respectively).

Note: Refer to the *ADCC Bioassay Effector Cells, Propagation Model Technical Manual,* TM385, for cell handling instructions for ADCC Bioassay Effector Cells.

The procedure below illustrates the use of the mTNFα Target Cells in the ADCC Reporter Bioassay to test two anti-TNFα antibody samples against a reference sample, in a single assay run using the mTNFα Target Cells, Propagation Model. Each test and reference antibody is run in triplicate, in a 10-point dilution series, in a single 96-well assay plate using the inner 60 wells. Other experimental and plate layouts are possible but may require further optimization.

Note: When preparing test and reference antibodies, choose an appropriate starting concentration and dilution scheme to achieve a complete dose-response curve with proper upper and lower asymptotes and sufficient points on the slope. For reference, we use 4.0µg/ml as a starting concentration (1X) and threefold serial dilution when testing infliximab.

5.A. Preparing ADCC Assay Reagents

1. **mTNFα Target Cell Plating Medium:** On the day before the assay, prepare an appropriate amount of cell plating medium (90% Ham's F-12/10% FBS). Thaw the FBS overnight at 4°C or in a 37°C water bath on the day of use. Mix well and warm to 37°C before use. For reference, 30ml of cell plating medium is typically sufficient for 120 wells in a 96-well assay format using the inner 60 wells.

2. **ADCC Assay Buffer:** On the day of the assay, prepare an appropriate amount of ADCC assay buffer (96% RPMI 1640/4% super low IgG FBS). Mix well and warm to 37°C before use. For reference, 30ml of ADCC assay buffer is typically sufficient for 120 wells in a 96-well assay format using the inner 60 wells.

   Note: The recommended ADCC assay buffer contains 4% super low IgG FBS. This concentration and type of FBS works well for the anti-TNFα antibody that we tested. If you experience assay performance issues when using this ADCC assay buffer, we recommend testing different serum concentrations and types, in the range of 0.5–10%.

3. **Bio-Glo™ Luciferase Assay Reagent:** For reference, 10ml of Bio-Glo™ Reagent is sufficient to assay 120 wells in a 96-well assay format. Thaw the Bio-Glo™ Luciferase Assay Buffer at 4°C 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 all of 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 and store the reconstituted Bio-Glo™ Reagent at ambient temperature (22–25°C) protected from light, before adding to assay plates.

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

   Note: The ADCC Reporter Bioassay is compatible only with the Bio-Glo™ Luciferase Assay System. **Do not** use the Bio-Glo-NL™ Luciferase Assay System with the ADCC Reporter Bioassay.

4. **Test and Reference Samples:** Using ADCC assay buffer as the diluent, prepare stock starting dilutions (dilu1, 2X final concentration) of two test antibodies (200µl each) and one reference antibody (400µl) in 1.5ml microcentrifuge tubes. Store the tubes containing antibody starting dilutions appropriately before making antibody serial dilutions.

   Note: If you are using infliximab (10mg/ml stock) as a reference antibody in your assay, prepare a 200µg/ml working stock of anti-TNFα antibody infliximab by adding 4µl of infliximab stock (10mg/ml) to 196µl of ADCC assay buffer. Prepare 400µl starting dilution of 12µg/ml of infliximab (dilu1, 3X final concentration) by adding 24µl of infliximab working stock to 376µl of ADCC assay buffer.

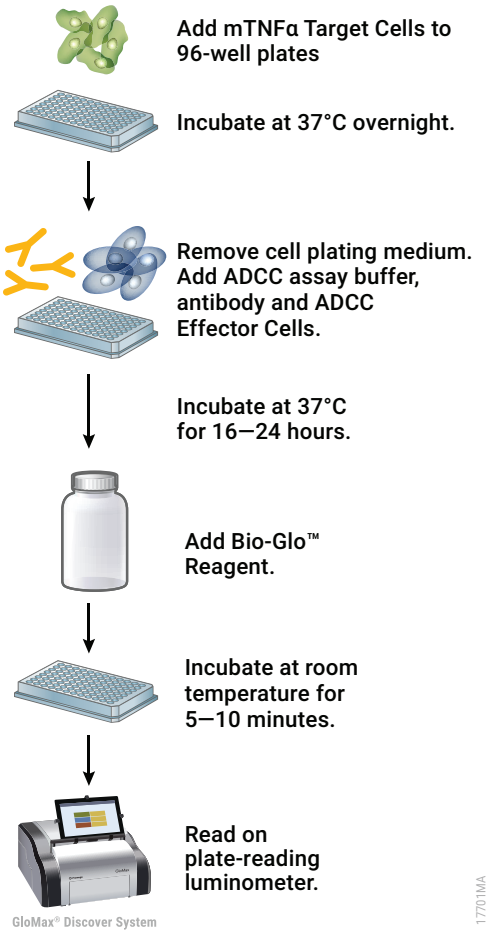

Figure 6. Schematic protocol for the ADCC Reporter Bioassay with mTNFα Target Cells, Propagation Model.

5.B. Plate Layout Design

For the protocol described here, use the plate layout illustrated in Figure 7 as a guide. The protocol describes serial replicate dilutions (n = 3) of test and reference antibody to generate two 10-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 7. Example plate layout showing non-clustered sample locations of test antibody and reference antibody dilution series and wells containing ADCC assay buffer (denoted by "B") alone.

5.C. Preparing and Plating mTNFα Target Cells

While maintaining the mTNFα Target Cells, follow the recommended cell seeding density (refer to Section 4 for culture instructions). Changes in cell culture volume or seeding density will affect cell growth rate and assay performance. Do not allow the cells to grow to 100% confluence. Only use the cells in the assay after the cell doubling rate has stabilized during propagation.

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

1. We recommend passaging the mTNFα Target Cells two days before plating for the assay (as described in Section 4) to ensure optimal and consistent assay performance.
2. On the day before performing the assay, prepare mTNFα Target Cell plating medium (Ham's F-12/10% FBS).
3. Aspirate the cell culture medium from the mTNFα Target Cells and wash with DPBS.
4. 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.

5. Add 8ml of mTNFα Target Cell plating medium to the flask. Transfer the cell suspension to a 50ml (or larger) conical centrifuge tube.
6. Gently mix and count the mTNFα Target Cells by Trypan blue staining.
7. Centrifuge at 150 × *g* for 10 minutes.
8. Gently resuspend the cell pellet in cell plating medium to achieve a concentration of 1×10^5 viable cells/ml.
9. 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. The final cell number in each well should be 1×10^4 cells/well.
10. Add 100µl of mTNFα Target Cell plating medium to each of the outside wells of the assay plates.
11. Place lids on the assay plates and incubate in a 37°C, 5% CO_2 incubator overnight (18–22 hours).

5.D. Preparing Antibody Serial Dilutions

The instructions described here are for preparing 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 400µl total 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: The instructions below use infliximab; follow the instructions below to prepare threefold serial dilutions. A threefold serial dilution for test antibodies is listed as an example below as well.

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

1. On the assay day, prepare an appropriate amount of ADCC assay buffer as described in Section 5.A.
2. 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 (see Figure 8).
3. Add 200µl of test antibody 1 and 2 starting dilutions (dilu1, 3X final concentration) to wells E11 and G11, respectively (see Figure 8).
4. Add 120µl of ADCC assay buffer to other wells in these four rows, from column 10 to column 2.
5. Transfer 60µl of the antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
6. 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 120µl of ADCC assay buffer without antibody as a negative control.
7. Cover the antibody dilution plate with a lid and keep at ambient temperature (22–25°C) while preparing the ADCC Effector Cells.

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 8. Example plate layout showing antibody serial dilutions.

5.E. Preparing ADCC Effector Cells

While maintaining the ADCC Effector Cells, 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 cell viability is greater than 95%.

1. Passage the cells two days before performing the assay.
2. Count the ADCC Effector Cells by Trypan blue staining and calculate the cell density and viability.
3. Transfer an appropriate amount of ADCC Effector Cells from the culture vessel to a 50ml conical tube or larger-sized centrifuge tube.
4. Pellet the cells at 130 × *g* for 10 minutes at ambient temperature and resuspend the pellet in ADCC assay buffer at 70% of the full volume needed to generate the targeted final cell density of 3×10^6 cells/ml, giving a final density of 7.5×10^4 cells/25µl/well.
5. Count the cells again and adjust the volume of ADCC assay buffer to achieve a final cell density of 3×10^6 cells/ml. You will need at least 3ml of ADCC Effector Cells to fill 120 assay wells, or the inner 60 wells of two assay plates.

5.F. Adding ADCC Assay Reagents and Cells to Plates

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

1. Take the 96-well assay plates containing mTNFα Target Cells out of the incubator. Invert the assay plate to remove the medium. Then, place the inverted plate on paper towels for 5–10 seconds to drain any remaining medium. Alternatively, remove 95µl of medium from each of the wells using a manual multichannel pipette.
2. Using a multichannel pipette, add 25µl of ADCC assay buffer to the inner 60 wells of both 96-well assay plates.
3. Using a multichannel pipette, add 25µl of the appropriate antibody dilution (Figure 8) to the assay plates according to the plate layout in Figure 7.
4. Mix the ADCC Effector Cells by inverting the tube and transfer the 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 the assay plates. Gently swirl the assay plates to ensure mixing of the Effector Cells and antibody.
5. Add 75µl of ADCC assay buffer to each of the outside wells of the assay plates.
6. Cover the assay plates with lids and incubate in a 37°C, 5% CO_2 incubator for 16–24 hours.

   Note: The 16–24 hour assay time was optimized using infliximab. We recommend optimizing assay time (5–24 hours) with your own antibody or other biologic samples.

5.G. Adding Bio-Glo™ Luciferase Assay Reagent

Note: The ADCC Reporter Bioassay is compatible only with the Bio-Glo™ Luciferase Assay System. **Do not** use the Bio-Glo-NL™ Luciferase Assay System with the ADCC Reporter Bioassay.

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

1. Remove assay plates from the 37°C incubator and equilibrate to ambient temperature (22–25°C) on the bench for 15 minutes.
2. Using a manual multichannel pipette, add 75µl of Bio-Glo™ Reagent to the inner 60 wells of both assay plates; avoid creating any bubbles.
3. Add 75µl of Bio-Glo™ Reagent to wells B1, D1 and F1 in each assay plate to determine plate background.
4. Incubate at ambient temperature for 5–20 minutes.
5. Measure luminescence using a plate reader with glow-type luminescence reading capabilities.

5.H. 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. Assay Protocol for CDC Assay using mTNFα Target Cells

The procedure below illustrates the use of the mTNFα Target Cells in a CDC assay to test two anti-TNFα antibody samples against a reference sample, in a single assay run using Membrane TNFα Target Cells, Propagation Model format. Each test and reference antibody is run in triplicate, in a 10-point dilution series, in a single 96-well assay plate. Other experimental and plate layouts are possible but may require further optimization.

Note: When preparing test and reference antibodies, choose an appropriate starting concentration and dilution scheme to achieve a complete dose-response curve with proper upper and lower asymptotes and sufficient points on the slope. For reference, we use 6.67µg/ml as a starting concentration (1X) and twofold serial dilution when testing infliximab.

6.A. Preparing CDC Assay Reagents

1. **mTNFα Target Cell Plating Medium/CDC Assay Buffer:** On the day before the assay, prepare an appropriate amount of cell plating medium/CDC assay buffer (90% Ham's F-12/10% FBS). Thaw the FBS overnight at 4°C or in a 37°C water bath on the day of use. Mix well and warm to 37°C before use. For reference, 50ml of cell plating medium/CDC assay buffer is typically sufficient for 132 wells in a 96-well assay format (Figure 10). After plating mTNFα Target Cells, remaining CDC assay buffer can be stored at 4°C overnight for use on the day of the assay.

2. **CytoTox-Glo™ Reagent:** Prepare an appropriate amount of CytoTox-Glo™ Reagent on the day of the assay. Thaw the CytoTox-Glo™ Assay Buffer in a room-temperature water bath, and equilibrate to ambient temperature, protected from light. Thirty minutes prior to the end of the assay, transfer 5ml of buffer into one amber bottle containing the AAF-Glo™ Substrate and mix by inversion, until the substrate is thoroughly dissolved. For reference, 10ml of CytoTox-Glo™ Reagent is enough for 132 assay wells in a 96-well assay format.

   For optimal results, use freshly prepared CytoTox-Glo™ Reagent. Use within 12 hours if stored at room temperature. The CytoTox-Glo™ Reagent can be stored at 4°C for up to 7 days with no appreciable loss of performance. The CytoTox-Glo™ Reagent can be stored in single-use aliquots for up to 4 months at −70°C. Freezing and thawing will damage the reagent and should be avoided.

3. **Normal Human Serum Complement:** Follow manufacturer's instructions for storage, preparation and handling. The recommended assay conditions include 10% normal human serum complement. This concentration and type of complement works well for the anti-TNFα antibodies we tested. If you experience assay performance issues when using normal human serum complement, we recommend testing different concentrations in the range of 5–20%.

4. **Test and Reference Samples:** Using CDC assay buffer as the diluent, prepare stock starting dilutions (dilu1, 1.5X final concentration) of two test antibodies (400µl each) and one reference antibody (800µl) in 1.5ml tubes. Store the tubes containing antibody starting dilutions appropriately before making antibody serial dilutions.

   Note: If you are using infliximab (10mg/ml stock) as a reference antibody in your assay, prepare a 200µg/ml working stock of infliximab by adding 4µl of infliximab stock (10mg/ml) to 196µl of CDC assay buffer. Prepare 800µl starting dilution of 10µg/ml of infliximab (dilu1, 1.5X final concentration) by adding 40µl of infliximab working stock to 760µl of CDC assay buffer.

6.A. Preparing CDC Assay Reagents (continued)

5. **Digitonin:** Prior to completion of the assay, prepare 1mg/ml of digitonin in CytoTox-Glo™ Cytotoxicity Assay Buffer from 20mg/ml of stock provided in the CytoTox-Glo™ Cytotoxicity Assay (Cat.# G9290) as follows: Combine 5µl of digitonin stock with 95µl of CytoTox-Glo™ Assay Buffer.

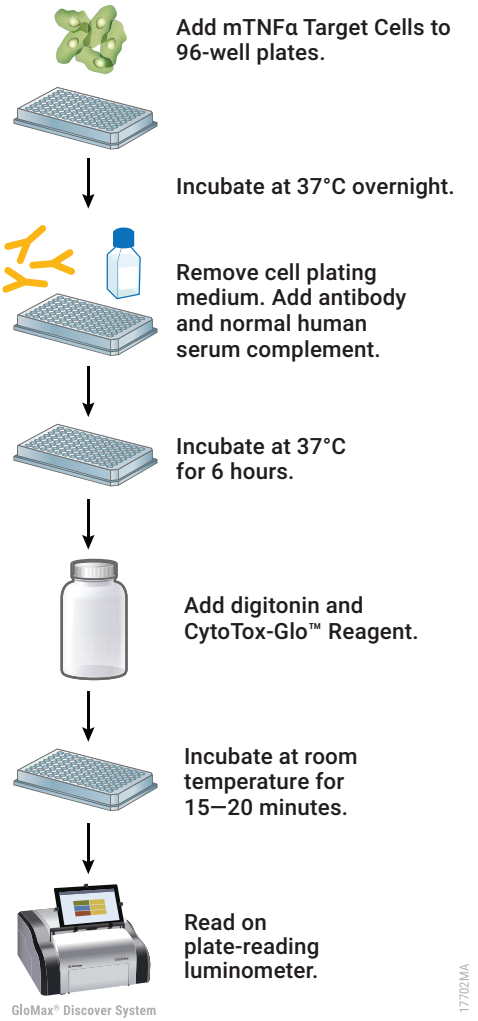

Figure 9. Schematic protocol for the CDC assay with Membrane TNFα Target Cells, Propagation Model.

6.B. Plate Layout Design

For the protocol described here, use the plate layout illustrated in Figure 10 as a guide. The protocol describes serial replicate dilutions (n = 3) of test and reference antibody to generate two 10-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	D	Reference Ab
C	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	D	Test Ab
D	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	D	Reference Ab
E	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	D	Test Ab
F	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	D	Reference Ab
G	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	D	Test Ab
H	B	B	B	B	B	B	B	B	B	B	B	B	Assay Buffer (B)

Figure 10. Example plate layout showing non-clustered sample locations of test antibody and reference antibody dilution series and wells containing digitonin (D) or CDC assay buffer alone (B).

6.C. Preparing and Plating mTNFα Target Cells

While maintaining the mTNFα Target Cells, follow the recommended cell seeding density (refer to Section 4 for culture instructions). Changes in cell culture volume or seeding density will affect cell growth rate and assay performance. Do not allow the cells to grow to 100% confluence. Only use the cells in the assay after the cell doubling rate has stabilized during propagation.

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

1. We recommend passaging the mTNFα Target Cells two days before plating for the assay (described in Section 4) to ensure optimal and consistent assay performance.
2. On the day before the assay, prepare an appropriate amount of mTNFα Target Cell plating medium (90% Ham's F-12/10% FBS).

3. Aspirate the cell culture medium from the mTNFα Target Cells and wash with DPBS.
4. 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.
5. Add 8ml of mTNFα Target Cell plating medium to the flask. Transfer the cell suspension to a 50ml (or larger) conical centrifuge tube.
6. Gently mix and count the mTNFα Target Cells by Trypan blue staining.
7. Centrifuge at 150 × *g* for 10 minutes.
8. Gently resuspend the cell pellet in cell plating medium to achieve a concentration of 1×10^5 viable cells/ml.
9. Transfer the suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 100µl of the cell suspension to wells B2 through G12 of a 96-well white flat-bottom assay plate (Figure 10). The final cell number in each well should be 1×10^4 cells/well.
10. Add 100µl of cell plating medium to each of the empty wells of the assay plates (Figure 10).
11. Place lids on the assay plates and incubate in a 37°C, 5% CO_2 incubator overnight (18–24 hours).

6.D. Preparing Antibody Serial Dilutions

The instructions described here are for preparing single stocks of twofold serial dilutions of a single antibody for analysis in triplicate (200µl 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 800µl of reference antibody at 1.5X the highest antibody concentration in your dose-response curve. You will need 400µl of each test antibody at 1.5X the highest antibody concentration in each of the test antibody dose-response curves. For other dilution schemes, adjust the volumes accordingly.

Note: The instructions below use infliximab; 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. On the day of the assay, warm CDC assay buffer prepared the day before to 37°C. Otherwise, prepare an appropriate amount of CDC assay buffer as described in Section 6.A.
2. To a sterile 12-well reservoir labeled reference, add 800µl of appropriate reference antibody starting dilution (dilu1, 1.5X final concentration) to well 11 (Figure 11).
3. To two additional sterile 12-well reservoirs labeled test 1 and test 2, add 400µl of test 1 and 2 antibody starting dilutions (dilu1, 1.5X final concentration) to well 11 (Figure 11).
4. For reference antibody, add 400µl of CDC assay buffer to other wells, 2 through 10, and 12 (well 1 is empty).
5. For test antibodies 1 and 2, add 200µl of CDC assay buffer to other wells, 2 through 10, and 12 (well 1 is empty).
6. Transfer 400µl of the reference antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
7. Repeat equivalent reference antibody twofold serial dilutions across the columns from right to left through column 3. Do not dilute into column 2.

Note: Well 2 contains 400µl of CDC assay buffer without antibody, as a negative control. Well 12 contains 400µl of CDC assay buffer without antibody and will be used for digitonin addition at end of the assay.

8. Transfer 200µl of the test 1 and 2 antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.

9. Repeat equivalent test 1 and 2 antibody twofold serial dilutions across the columns from right to left through column 3. Do not dilute into column 2.

   Note: Well 2 contains 200µl of CDC assay buffer without antibody, as a negative control. Well 12 contains 200µl of CDC assay buffer without antibody and will be used for digitonin addition at end of assay.

10. Cover the antibody dilution reservoirs with their lids and keep at ambient temperature (22–25°C) while preparing the normal human serum complement.

Recommended 12-well Reservoir Layouts for Antibody Dilutions Prepared from a Single Antibody Stock.

1	2	3	4	5	6	7	8	9	10	11	12	
	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	no Ab	Reference Ab

1	2	3	4	5	6	7	8	9	10	11	12	
	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	no Ab	Test Ab 1

1	2	3	4	5	6	7	8	9	10	11	12	
	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	no Ab	Test Ab 2

Figure 11. Example 12-well reservoir layouts showing antibody serial dilutions.

6.E. Preparing Normal Human Serum Complement

Note: Normal human serum complement is heat labile and care must be taken to ensure it is fully intact when used in the assay. Follow manufacturer's instructions for storage and handling.

1. Dilute the normal human serum complement with CDC assay buffer, to achieve a 30% solution. Once diluted in the assay plate, this gives a 10% final concentration. You will need at least 4ml of 30% complement to fill 120 assay wells, or 60 wells of two assay plates.

6.F. Adding Antibody and Complement to Assay Plates

1. Remove the 96-well assay plates containing mTNFα Target Cells from the incubator. Invert the assay plate to remove the medium. Then, place the inverted plate on paper towels for 5–10 seconds to drain any remaining medium. Alternatively, remove 95µl of medium from each of the wells using a manual multichannel pipette.
2. Using a multichannel pipette, add 50µl of appropriate antibody dilution to the assay plates according to the plate layout in Figure 10.
3. Using a multichannel pipette, add 25µl of the 30% normal human serum complement to each of the inner 60 wells of the assay plates (Figure 10).
4. Using a multichannel pipette, add 25µl of CDC assay buffer to wells B12–G12 (digitonin wells).
5. Add 75µl of CDC assay buffer to each of the empty outside wells of the assay plates.
6. Cover the assay plates with lids and incubate in a 37°C, 5% CO_2 incubator for 6 hours.

   Note: The 6-hour assay time was optimized using infliximab. We recommend optimizing assay time (3–24 hours) with your own antibody or other biologic samples.

6.G. Adding Digitonin and CytoTox-Glo™ Reagent

1. During the 6-hour incubation, reconstitute the CytoTox-Glo™ Cytotoxicity Assay Reagent according to the instructions in Section 6.A.
2. At the end of the 6-hour incubation, remove the assay plates from the incubator and immediately add 8µl of 1mg/ml digitonin (prepared in Section 6.A) to wells B12–G12, per the plate layout shown in Figure 10.
3. Gently shake the plate briefly to mix the digitonin in the well.
4. Allow plate to equilibrate to ambient temperature (15–20 minutes).
5. Add 40µl per well of CytoTox-Glo™ Reagent (at ambient temperature) to all wells containing mTNFα Target Cells (wells B2–G12).
6. Add 40µl per well of CytoTox-Glo™ Reagent to wells B1, D1, and F1 to determine plate background.
7. Incubate at ambient temperature for 15 minutes.
8. Measure luminescence using a plate reader with glow-type luminescence reading capabilities.

6.H. Data Analysis

1. Determine the plate background by calculating the average RLU from wells B1, D1 and F1.
2. Determine the maximum cytotoxicity by calculating the average RLU from wells B12–G12.
3. Calculate Fold Induction:

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

4. Calculate Percent Specific Lysis:

$$\text{Percent Specific Lysis} = \frac{\text{RLU (antibody} - \text{background)}}{\text{RLU (digitonin} - \text{background)}} \times 100$$

5. Graph data as RLU versus Log_{10}[antibody], Fold Induction versus Log_{10}[antibody] and Percent Specific Lysis 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.

7. 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	Possible Causes and Comments
Low luminescence measurements (RLU readout)	Choose a sensitive instrument designed for luminescence detection. Instruments designed primarily for fluorescence detection are not recommended. Luminometers measure and report luminescence as relative values, and actual numbers 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™ Reagent or CytoTox-Glo™ Reagent leads to low RLU. Store and handle the reagents according to the instructions.
Weak CDC assay repsonse (low % lysis)	Optimize the normal human serum complement from 5–20% if CDC assay performance is not ideal.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Weak assay response (low fold induction)	Optimize the concentration range of your test sample(s) to achieve a complete dose response with complete upper and lower asymptotes. The EC_{50} values obtained in the ADCC Reporter Bioassay or CDC assay with mTNFα Target Cells may vary from the EC_{50} value obtained using other methods.
	Optimize the Super Low IgG FBS concentration from 0.5–10% in ADCC assay buffer if ADCC Reporter Bioassay performance is not ideal.
Variability in assay performance	Variations in cell growth conditions including cell plating, harvest density, cell viability and cell doubling time may cause low assay performance and high assay variation. 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.

8. References

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9. Appendix

9.A. Representative Assay Results

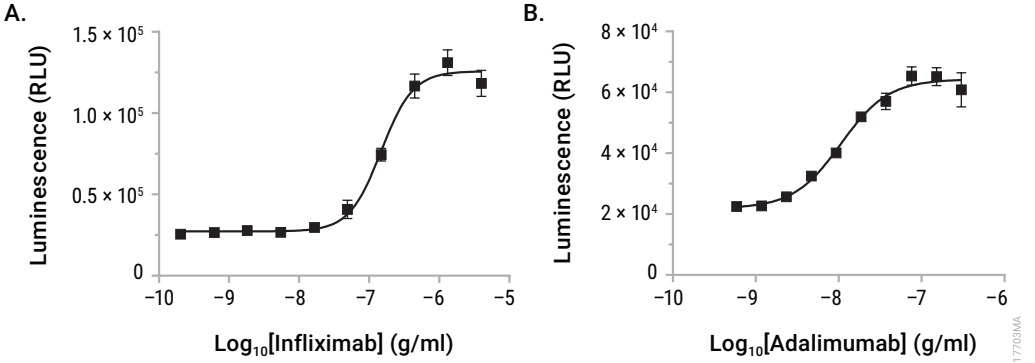

Figure 12. The ADCC Reporter Bioassay with mTNFα Target Cells measures the activity of the anti-TNFα antibody infliximab and adalimumab. mTNFα Target Cells were added to a 96-well assay plate 18-hours prior to the assay. On the day of assay, ADCC Effector Cells and a titration of infliximab (**Panel A**) or adalimumab (**Panel B**) were added. After a 24-hour induction at 37°C, Bio-Glo™ 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 EC_{50} was 148ng/ml for infliximab and 10.7ng/ml for adalimumab. Fold induction was 4.9 and 2.9 for infliximab and adalimumab, respectively. Data were generated using cell propagation model cells.

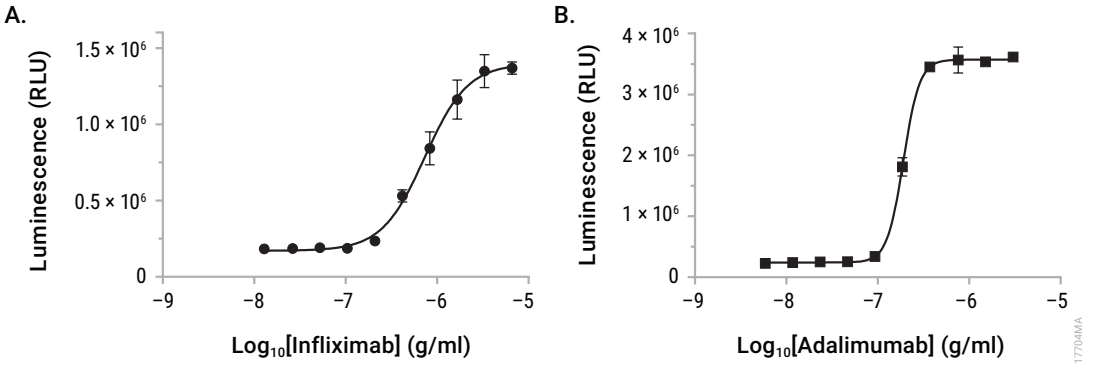

Figure 13. A CDC assay measures the activity of the anti-TNFα antibody infliximab and adalimumab. mTNFα Target Cells were added to a 96-well assay plate 18-hours prior to the assay. On the day of assay, 10% normal human serum complement and a titration of infliximab (**Panel A**) or adalimumab (**Panel B**) were added. After a 6-hour induction at 37°C, CytoTox-Glo™ 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 EC_{50} for infliximab was 0.73µg/ml, the fold-induction was 7.7 and the percent specific lysis was 40.2%. The EC_{50} for adalimumab was 0.19µg/ml, the fold-induction was 15.7 and the percent specific lysis was 37.1%. Data were generated using propagation model cells.

9.B. Composition of Buffers and Solutions

initial cell culture medium for mTNFα Target Cells

90% Ham's F-12
10% FBS

cell growth medium for mTNFα Target Cells

90% Ham's F-12
10% FBS
500µg/ml Geneticin™ antibiotic (G418)

cell plating medium for mTNFα Target Cells

90% Ham's F-12
10% FBS

cell freezing medium for mTNFα Target Cells

85% Ham's F-12
10% FBS
5% DMSO

ADCC assay buffer

96% RPMI 1640 with L-glutamine and HEPES
4% Super Low IgG FBS

CDC assay buffer

90% Ham's F-12
10% FBS

9.C. 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 VEGF Target Cells**	1 each	J3351
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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9.C. 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 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

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.

Control Antibodies and Proteins

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

Detection Reagent

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 through Promega Elite Access. To view and order Promega Bioassay products visit: **www.promega.com/products/reporter-bioassays/** or e-mail: **eliteaccess@promega.com**

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