

TECHNICAL MANUAL

CD28 Bioassay, Propagation Model

Instructions for Use of Product
JA1072

CD28 Bioassay, 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

1. Description	2
2. Product Components and Storage Conditions	8
3. Before You Begin	9
3.A. Materials to Be Supplied by the User	10
4. Preparing CD28 Effector Cells	11
4.A. Cell Thawing and Initial Cell Culture	11
4.B. Cell Maintenance and Propagation	11
4.C. Cell Freezing and Banking	12
5. Preparing FcγRIIb aAPC/CHO-K1 Cells	12
5.A. Cell Thawing and Initial Cell Culture	12
5.B. Cell Maintenance and Propagation	13
5.C. Cell Freezing and Banking	13
6. Assay Protocol for FcγRIIb-Dependent or FcγRIIb-Independent Antibodies	14
6.A. Preparing Assay Buffer, Bio-Glo™ Reagent and Antibody Samples	14
6.B. Plate Layout Design	17
6.C. Preparing and Plating FcγRIIb aAPC/CHO-K1 Cells	17
6.D. Preparing Antibody Serial Dilutions	18
6.E. Preparing CD28 Effector Cells	19
6.F. Adding CD28 Effector Cells and Antibody to Assay Plates	20
6.G. Adding Bio-Glo™ Reagent	20
6.H. Data Analysis	20
7. Troubleshooting	21
8. References	22
9. Appendix	23
9.A. Representative Assay Results	23
9.B. Composition of Buffers and Solutions	24
10. Summary of Changes	24

1. Description

The human immune system is regulated by a complex network of inhibitory and stimulatory receptors that facilitate the elimination of pathogens, while maintaining tolerance to self-antigens. T cells play a central role in cell-mediated immunity against pathogens; however, T cells also contribute to the pathogenesis and exacerbation of autoimmune disorders.

Optimal activation of naive T cells is initiated by engagement of the T cell antigen receptor (TCR)/CD3 complex and the costimulatory receptor CD28. CD28 binds to the B7 family members CD80 and CD86 (collectively referred to as B7 in this Technical Manual) on antigen-presenting cells (APCs). Costimulation of T cells by CD28 activation initiates signaling cascades that result in AP-1 and NFκB transcription factor activation (1). These pathways significantly enhance T cell cytokine production—specifically, interleukin-2 (IL-2)—which promotes T cell proliferation, differentiation and survival (2).

Antibodies that agonize CD28 have the potential to enhance immune responses against cancer and chronic infection. Unfortunately, disastrous phase 1 clinical results have stalled the development of these molecules (3). Therefore, the use of CD28 agonists for clinical use has been limited. However, CD28 agonist antibodies may be important in the development of alternative therapeutic modalities.

In adoptive cell transfer immunotherapy, T cells are extracted from patients, modified and expanded ex vivo, and re-infused into the patient. The most common method of ex vivo expansion is through the use of CD3- and CD28-targeted agonistic antibodies. Therefore, these agonist antibodies are considered critical reagents and must undergo appropriate quality controls (4,5).

Quantitative functional bioassays are needed in the development of biologic drugs designed to activate CD28. Current methods rely on primary human T cells with APCs, and measurement of functional endpoints such as cell proliferation, cell surface marker expression and cytokine production. 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 a quality-controlled drug development setting.

The CD28 Bioassay, Propagation Model^(a-d) (Cat.# JA1072), 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 potential biologics that activate CD28 (6). The bioassay consists of two genetically engineered cell lines:

- **CD28 (TCR/CD3) Effector Cells:** Jurkat T cells endogenously expressing TCR, CD3 and CD28 receptors and engineered with a luciferase reporter driven by a CD28 pathway-dependent promoter.
- **FcγRIIb aAPC/CHO-K1 Cells^(b):** CHO-K1 cells engineered to express FcγRIIb that serves as a crosslinking receptor, which binds to antibody Fc domains and clusters agonist antibodies for maximal activity, and an engineered cell-surface protein designed to activate the T cell receptor (TCR) complex in an antigen-independent manner.
Note: Alternatively, aAPC/CHO-K1 Cells (available separately, Cat.# J3312) engineered with the cell-surface protein designed to activate the TCR complex in an antigen-independent manner, can be substituted for use with FcγRIIb-independent antibodies or other biologics that do not require crosslinking for optimal activity.

The CD28 Effector Cells and FcγRIIb aAPC/CHO-K1 Cells are provided in Cell Propagation Model (CPM) format, which includes cryopreserved cells that can be thawed, propagated and banked for long-term use.

In addition to the CD28 Bioassay, we offer Control Ab, Anti-CD28 (Cat.# K1231), for use as a positive control for assay optimization and routine quality control. When screening for agonist antibodies of CD28, we recommend performing the assay with the provided FcγRIIb aAPC/CHO-K1 Cells in parallel with aAPC/CHO-K1 Cells to determine the role of FcγRIIb-mediated crosslinking in enhancing the effect of agonist antibodies.

Inducing the CD28 Effector Cells with a CD28 agonist antibody results in increased promoter-mediated luminescence (Figure 1). The bioluminescent signal is quantified using the Bio-Glo™ Luciferase Assay System, and a standard luminometer such as the GloMax® Discover System.

Blockade of CD28 has proven beneficial as a separate immunotherapy strategy in preclinical and clinical studies to reduce autoimmunity and alloimmunity (7,8). The CD28 Bioassay is not designed to detect blocking activity of CD28 antibodies. We offer separately the CD28 Blockade Bioassay (Cat. # JA6101) for screening and potency testing of CD28 antagonists.

The CD28 Bioassay reflects the mechanism of action (MOA) of biologics designed to activate CD28. Specifically, CD28 activation-mediated luminescence is increased following the addition of a CD28 activating antibody but not following addition of anti-ICOS or anti-4-1BB agonist antibodies (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 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 4). In addition, the bioassay can be performed in the presence of human serum (in antibody samples), indicating potential for further development into a neutralizing antibody bioassay (Figure 5).

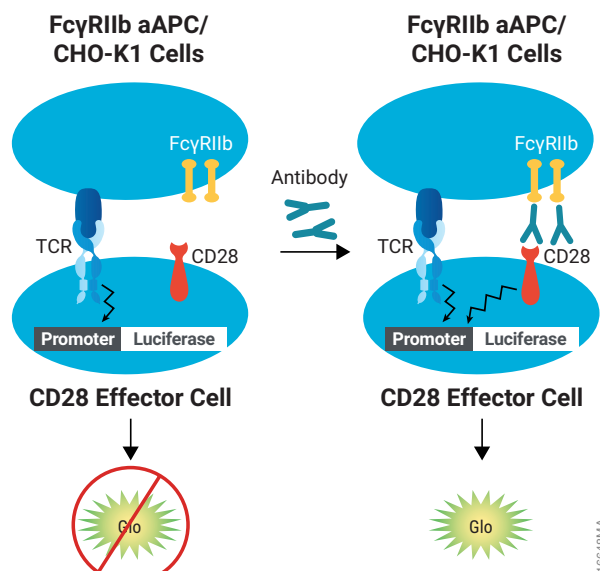


Figure 1. Representation of the CD28 Bioassay. The CD28 Bioassay for FcγRIIb-dependent agonist antibodies consists of two cell lines, CD28 Effector Cells and FcγRIIb aAPC/CHO-K1 Cells. In the absence of agonist antibody, CD28 is not activated and the luminescence signal is low. In the presence of FcγRIIb aAPC/CHO-K1 Cells, anti-CD28 antibody can be crosslinked, thereby enhancing induction of CD28 pathway-activating luminescence in a dose-dependent manner. CD28 Effector Cells can also be used for antibodies that are FcγRIIb-independent by substituting aAPC/CHO-K1 Cells.

1. Description (continued)

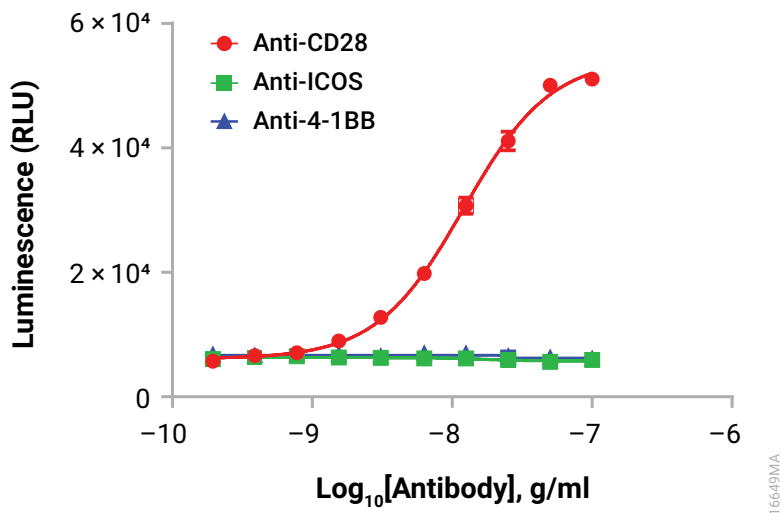


Figure 2. The CD28 Bioassay reflects the mechanism of action (MOA) and specificity of biologics designed to activate CD28. CD28 Effector Cells were incubated with FcγRIIb aAPC/CHO-K1 Cells in the presence of serial titrations of agonist antibodies as indicated. After a 5-hour incubation, Bio-Glo™ Reagent 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. The CD28 Bioassay Shows Precision, Accuracy and Linearity.

Parameter	Results	
Accuracy	% Expected Relative Potency	% Recovery
	50	43.6
	70	67.2
	100	93.5
	140	139.9
	200	205.5
Repeatability (% CV)	100% (Reference)	4.9
Intermediate Precision (% CV)		6.3
Linearity (r^2)		0.998
Linearity ($y = mx + b$)		$y = 1.075x - 10.48$
A 50–200% theoretical potency series of Control Ab, Anti-CD28, was analyzed in triplicate in three independent experiments performed on three days by two analysts using the CD28 Bioassay for FcγRIIb-dependent antibodies. Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were analyzed and relative potencies were calculated after parallelism determination using JMP® software. Data were generated using thaw-and-use cells.		

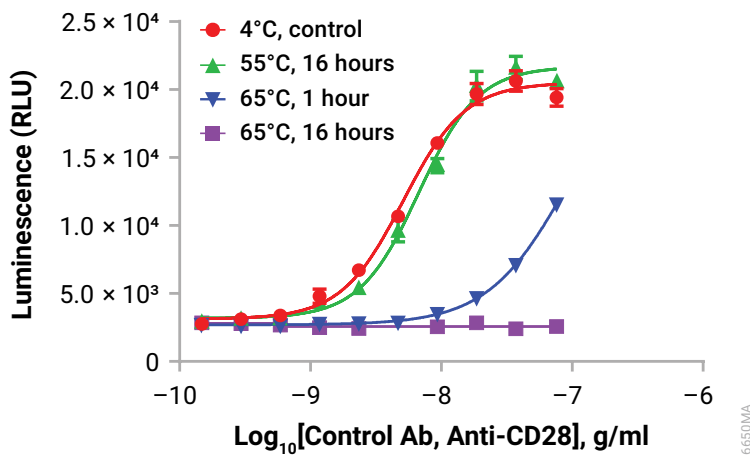


Figure 3. The CD28 Bioassay is stability-indicating. Samples of Control Ab, Anti-CD28 were maintained at 4°C (control) or heat-treated at the indicated times and temperatures, then analyzed using the CD28 Bioassay protocol for FcγRIIb-dependent antibodies. Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software and generated using thaw-and-use cells.

1. Description (continued)

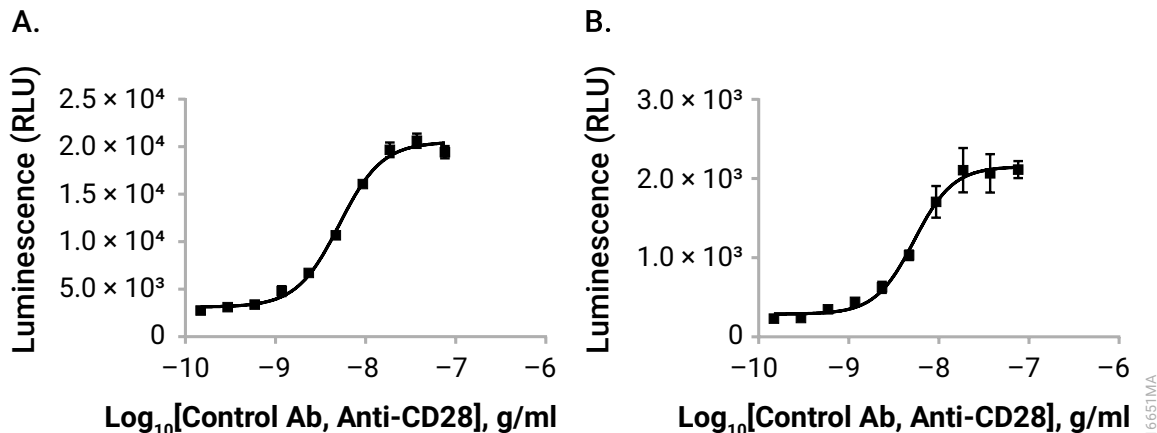


Figure 4. The CD28 Bioassay is amenable to 384-well plate format. Panel A. The CD28 Bioassay for FcγRIIb-dependent antibodies was performed in 96-well plates as described in this technical manual with a titration of Control Ab, Anti-CD28. **Panel B.** The CD28 Bioassay protocol for FcγRIIb-dependent antibodies was performed in 384-well format as briefly described here. FcγRIIb aAPC/CHO-K1 Cells were harvested and 8×10^3 cells/20μl/well plated 16–24 hours prior to assay, in a 384-well white assay plate (e.g., Corning® Cat. # 3570). On the day of the assay, spent media was removed from the 384-well plate immediately prior to plating and 8μl of 2X serially diluted Control Ab, Anti-CD28, was added, followed by the addition of 2×10^4 cells/8μl/well of CD28 Effector Cells. After the 5-hour assay induction at 37°C, 5% CO₂, 16μl/well of 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. The EC₅₀ values were 5.1 and 5.3ng/ml for the 96- and 384-well formats, respectively, and the fold induction was 7.4 and 9.4 for the 96- and 384-well formats, respectively. Data were generated using thaw-and-use cells.

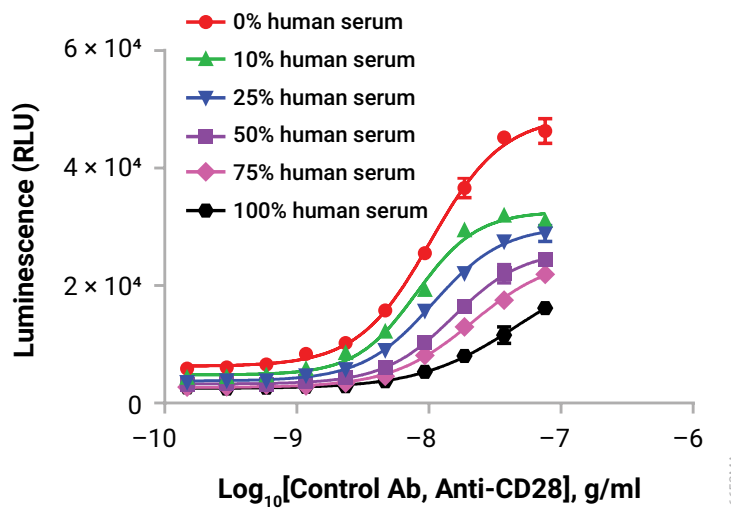


Figure 5. The CD28 Bioassay is tolerant to human serum. Following the CD28 Bioassay protocol for FcγRIIb-dependent antibodies, Control Ab, Anti-CD28, was analyzed in the absence or presence of pooled normal human serum (0–100% in the antibody sample), resulting in final assay concentration of human serum (0–50%). After the 5-hour assay induction at 37°C, 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. The CD28 Bioassay is tolerant to the presence of human serum with this serum pool. A different human serum pool showed similar effects on the assay (data not shown).



2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
CD28 Bioassay, Propagation Model	1 each	JA1072

Not for Medical Diagnostic Use. Includes:

- 2 vials TCR/CD3 Effector Cells (IL-2, CPM), (CD28 Effector Cells) 2×10^7 cells/ml (1.0ml per vial)
- 2 vials FcγRIIb aAPC/CHO-K1 Cells (CPM), 1.2×10^7 cells/ml (1.0ml per vial)

Notes:

- CD28 Effector Cells are labeled TCR/CD3 Effector Cells (IL-2). Please note the vial label when placing the vials into storage.
- Thaw and propagate one vial to create frozen cell banks before use in an assay. The second vial 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 negatively impact cell viability and cell performance.

The CD28 Effector Cells can also be paired with aAPC/CHO Cells for antibodies that are FcγRIIb-independent. The aAPC/CHO-K1 Cells, Propagation Model, is available separately as a standalone product.

PRODUCT AVAILABLE SEPARATELY	SIZE	CAT.#
aAPC/CHO-K1 Cells, Propagation Model	1 each	J3312

Not for Medical Diagnostic Use. Includes:

- 2 vials aAPC/CHO-K1 Effector Cells (CPM), 1.2×10^7 cells/ml (1.0ml per vial)

Note: Thaw and propagate one vial to create frozen cell banks before use in an assay. The second vial 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 negatively impact cell viability and cell performance.

3. Before You Begin

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

Note the catalog number and 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 Certificate of Analysis.

Note: The CD28 Bioassay uses the Bio-Glo™ Luciferase Assay System (Cat.# G7940, G7941) for detection.

The CD28 Bioassay, Propagation Model, is intended to be used with user-provided antibodies or other biologics designed to activate CD28. Control Ab, Anti-CD28 (Cat.# K1231), and aAPC/CHO-K1 Cells (Cat.# J3312) are available separately for use in assay optimization and routine quality control. We strongly recommend including Control Ab, Anti-CD28, along with the provided FcγRIIb aAPC/CHO-K1 Cells as a positive control in the first few assays to gain familiarity with the assay. Data generated using Control Ab, Anti-CD28, are shown in Section 9.A, Representative Assay Results.

Cell thawing, propagation and banking should be performed exactly as described in Section 4 and 5. 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 this technical manual were established using Control Ab, Anti-CD28, with FcγRIIb aAPC/CHO-K1 Cells or aAPC/CHO-K1 Cells. You may need to adjust the parameters provided here and optimize assay conditions for your own antibody or biologic samples.

The CD28 Bioassay 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. 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 light unit (RLU) readings will vary with the sensitivity and settings of each instrument. If using a reader with adjustable gain, we recommend a high gain setting. The use of different instruments and gain adjustment will affect the magnitude of the raw data, but should not affect the measured relative potency of test samples.

3.A. Materials to Be Supplied by the User

(Composition of Buffers and Solutions is provided in Section 9.B.)

Reagents

- user-defined anti-CD28 antibodies or other biologics samples
- RPMI 1640 Medium with L-glutamine and HEPES (e.g., Corning® Cat.# 10-041-CV or GIBCO® Cat.# 22400105)
- fetal bovine serum (e.g., VWR Cat.# 89510-194, GIBCO® Cat.# 35-015-CV or HyClone Cat.# SH30071.03)
- hygromycin B (e.g., GIBCO® Cat.# 10687 010)
- sodium pyruvate (e.g., GIBCO® Cat.# 11360 070)
- MEM nonessential amino acids, 100X (e.g., GIBCO® Cat.# 11140 050)
- DMSO (e.g., Sigma Cat.# D2650)
- Trypan blue solution (e.g., Sigma Cat.# T8154)
- Bio-Glo™ Luciferase Assay System (Cat.# G7940, G7941)
- Ham's F-12 Medium with L-glutamine (e.g., GIBCO® Cat.# 11765062) for plating CHO-K1 Cells
- Accutase® solution (e.g., Sigma Cat.# A6964) for lifting CHO-K1 Cells
- DPBS (e.g., GIBCO® Cat.# 14190) for washing CHO-K1 Cells
- **optional:** Control Ab, Anti-CD28 (Cat.# K1231). This antibody is FcγRIIb-dependent and requires the use of FcγRIIb aAPC/CHO-K1 Cells for optimal signal.
- **optional:** aAPC/CHO-K1 Cells if using FcγRIIb-independent antibodies (Cat.# J3312)

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., Costar®/Corning® Cat.# 4870)
- 37°C, 5% CO₂ incubator
- 37°C water bath
- sensitive plate reader with glow luminescence measuring capability or luminometer (e.g., GloMax® Discover System or equivalent system)

4. Preparing CD28 Effector 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 40ml of initial cell culture medium by adding 4ml of FBS to 36ml of RPMI 1640 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 CD28 Effector Cells, labeled TCR/CD3 Effector Cells (IL-2), from storage at -140°C and thaw in a 37°C water bath with gentle agitation (no inversion) 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 $90 \times g$ for 10 minutes.
6. Carefully aspirate the medium, and resuspend the cell pellet in 25ml of prewarmed initial cell culture medium.
7. Transfer the cell suspension to a T75 tissue culture flask, and place the flask horizontally in a humidified 37°C, 5% CO₂ incubator.
8. Incubate for approximately 48 hours before passaging the cells.

4.B. Cell Maintenance and Propagation

Note: 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 after thawing, at which time cell viability is typically >95%, and the average cell doubling rate is 28–30 hours. Passage number should be recorded for each passage. In our experience, cells maintain their functionality for up to 25 passages, or 46 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 4.5×10^5 cells/ml if passaging every 2 days (e.g., Monday-Wednesday or Wednesday-Friday) or 2.5×10^5 cells/ml if passaging every 3 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 2×10^6 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% CO₂ incubator.

4.C. Cell Freezing and Banking

1. On the day of cell freezing, prepare fresh cell freezing medium and keep on ice.
2. Gently mix the cells with a pipette to create a homogeneous 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 5×10^6 – 2×10^7 cells/ml.
4. Transfer the cell suspension to 50ml sterile conical tubes or larger sized centrifuge tubes, and centrifuge at $130 \times g$, 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 5×10^6 – 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. Preparing FcγRIIb aAPC/CHO-K1 Cells



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

Note: The same conditions listed below for the preparation of FcγRIIb aAPC/CHO-K1 Cells are also recommended for preparation of the aAPC/CHO-K1 Cells (Cat.# J3312).

5.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 F-12 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 FcγRIIb aAPC/CHO-K1 Cells from storage at -140°C and thaw in a 37°C water bath with gentle agitation (no inversion) 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 $90 \times g$ 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 flasks and place the flasks horizontally in a humidified 37°C , 5% CO_2 incubator.
8. Incubate for approximately 24 hours before passaging the cells.

5.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. 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 and place the flasks in a humidified 37°C, 5% CO₂ incubator for 5–7 minutes or until the cells round up and detach from the bottom of the flasks.
3. Add 8ml of cell growth medium to each T75 flask. Transfer the cell suspension to a sterile 15ml or 50ml conical tube.
4. Count the cells by Trypan blue staining. We recommend seeding the cells at a density of 4×10^4 cells/cm² if passaging every 2 days (e.g., Monday-Wednesday or Wednesday-Friday) or 2×10^4 cells/cm² if passaging every 3 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% CO₂ incubator.

5.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 the flasks in a humidified 37°C, 5% CO₂ incubator for 5–7 minutes or until the cells round up and detach from the bottom of the flasks.
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 the desired cell freezing densities of 3×10^6 – 1×10^7 cells/ml.
6. Transfer the cell suspension to 50ml sterile conical tubes or larger-sized centrifuge tubes, and centrifuge at $180 \times g$, 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 –140°C or below for long-term storage.

6. Assay Protocol for FcγRIIb-Dependent or FcγRIIb-Independent Antibodies

This assay protocol requires two engineered cell lines: CD28 Effector Cells and FcγRIIb aAPC/CHO-K1 Cells for FcγRIIb-dependent antibodies or aAPC/CHO-K1 Cells available separately (Cat.# J3312) for FcγRIIb-independent antibodies. The FcγRIIb aAPC/CHO-K1 Cells and aAPC/CHO-K1 Cells are also available in thaw-and-use format (Cat.# JA9331, JA9335 and Cat.# JA9441, JA9445, respectively; see *CD28 Bioassay Technical Manual*, #TM633). Either CPM or thaw-and-use format can be used in this assay.

The procedure below illustrates the use of the CD28 Bioassay to test two FcγRIIb-dependent antibody samples against a reference sample in a single assay run using the FcγRIIb aAPC/CHO-K1 Cells, Propagation Model format (Figure 6). 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.

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 0.15μg/ml as a starting concentration (1X) and twofold serial dilution when testing Control Ab, Anti-CD28, using the FcγRIIb-dependent method. For the FcγRIIb-independent method, we used 1.5μg/ml as a starting concentration (1X) and a twofold serial dilution when testing Control Ab, Anti-CD28.

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

- FcγRIIb aAPC/CHO-K1 Cell Plating Medium:** On the day before the assay, prepare 40ml of cell plating medium (90% Ham's F-12/10% FBS) in a 50ml conical tube. Thaw the FBS overnight at 4°C or in a 37°C water bath on the day of use. Add 4ml of FBS to 46ml of Ham's F-12 medium. Mix well and warm to 37°C before use.
- Assay Buffer:** On the day of the assay, prepare an appropriate amount of assay buffer (90% RPMI 1640/10% FBS). Mix well and warm to 37°C before use. For reference, 30ml 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 10% FBS. This concentration of FBS works well for the Control Ab, Anti-CD28, that we 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™ 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 the 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. When stored appropriately, Bio-Glo™ reagent will maintain at least 80% activity after 24 hours at ambient temperature.

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 6 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. The Bio-Glo™ Reagent can be stored at room temperature after reconstitution with ~18% loss in luminescence after 24 hours.

4. **Test and Reference Samples:** Using assay buffer as the diluent, prepare starting dilutions (dilu1, 2X final concentration) of two test antibodies (300µl each) and one reference antibody (600µl) in 1.5ml tubes. Store the tubes containing antibody starting dilutions appropriately before making antibody serial dilutions.

Notes:

For **FcγRIIb dependent assay**, if you are using FcγRIIb aAPC/CHO-K1 Cells and Control Ab, Anti-CD28, as a reference antibody in your assay, prepare a 10µg/ml **working stock** of Control Ab, Anti-CD28, by adding 2µl of Control Ab, Anti-CD28 **stock** (1.0mg/ml) to 198µl of assay buffer. Prepare a 600µl starting dilution of 0.3µg/ml of Control Ab, Anti-CD28 (dilu1, 2X final concentration), by adding 18µl of Control Ab, Anti-CD28 **working stock** (10µg/ml) to 582µl of assay buffer. Store the antibody starting dilution on ice until ready to use in the assay.

For **FcγRIIb independent assay**, if you are using aAPC/CHO-K1 Cells and Control Ab, Anti-CD28, as a reference antibody in your assay, prepare a 10µg/ml **working stock** of Control Ab, Anti-CD28, by adding 2µl of Control Ab, Anti-CD28 **stock** (1.0mg/ml) to 198µl of assay buffer. Prepare a 600µl starting dilution of 3µg/ml of Control Ab, Anti-CD28 (dilu1, 2X final concentration), by adding 180µl of Control Ab, Anti-CD28 **working stock** (10µg/ml) to 420µl of assay buffer. Store the antibody starting dilution on ice until ready to use in the assay.

6.A. Preparing Assay Buffer, Bio-Glo™ Reagent and Antibody Samples (continued)

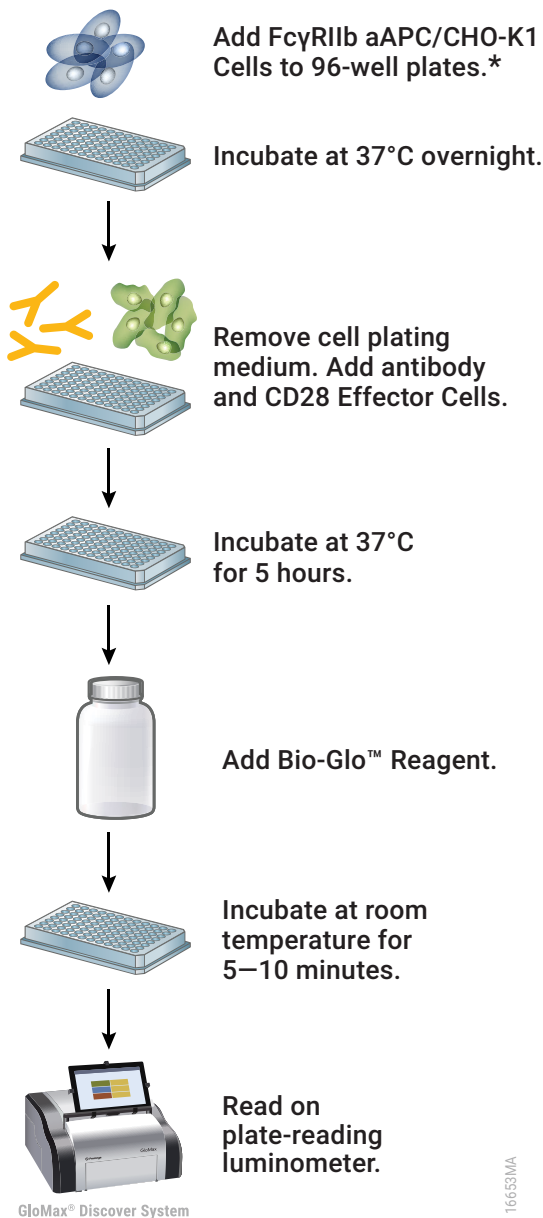


Figure 6. Schematic protocol for the CD28 Bioassay, Propagation Model, for FcγRIIb-dependent antibodies. *For FcγRIIb-independent antibodies, substitute aAPC/CHO-K1 Cells (Cat.# J3312).

6.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 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 7. Example plate layout showing nonclustered sample locations of test antibody and reference antibody dilution series and wells containing assay buffer (denoted by "B") alone.

6.C. Preparing and Plating FcγRIIb aAPC/CHO-K1 Cells

While maintaining the FcγRIIb aAPC/CHO-K1 Cells, follow the recommended cell seeding density (refer to Section 5 for culture instructions for FcγRIIb aAPC/CHO-K1 Cells, Propagation Model). 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 FcγRIIb aAPC/CHO-K1 Cells two days before plating for the assay (as described in Section 5) to ensure optimal and consistent assay performance.
2. On the day before performing the assay, prepare FcγRIIb aAPC/CHO-K1 cell plating medium (Ham's F-12/10% FBS) for the FcγRIIb aAPC/CHO-K1 Cells.

6.C. Preparing and Plating FcγRIIb aAPC/CHO-K1 Cells (continued)

3. Aspirate the cell culture medium from the FcγRIIb aAPC/CHO-K1 Cells and wash with DPBS.
4. Add 3ml of Accutase® solution to each T75 flask, and place the flasks in a 37°C, 5% CO₂ incubator for 5–7 minutes or until the cells round up and detach from the bottom of the flasks.
5. Add 7ml of FcγRIIb aAPC/CHO-K1 cell plating medium to the flasks. Transfer the cell suspension to a 50ml (or larger) conical centrifuge tube.
6. Gently mix and count the FcγRIIb aAPC/CHO-K1 Cells by Trypan blue staining.
7. Centrifuge at 230 × g for 10 minutes.
8. Gently resuspend the cell pellet in cell plating medium to achieve a concentration of 4 × 10⁵ 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 4 × 10⁴ cells/well.
10. Add 100μl of 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% CO₂ incubator overnight (18–22 hours).

6.D. Preparing Antibody Serial Dilutions

The instructions described here are for the preparation of a single stock of twofold serial dilutions of a single antibody for analysis in triplicate (150μl 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 600μl of reference antibody at 2X the highest antibody concentration in your dose-response curve. You will need 300μl of each test antibody at 2X 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-CD28, as a control in the assay, follow the instructions below to prepare twofold serial dilutions. A twofold serial dilution for test antibodies is listed as an example below as well.

1. On the day of the assay, prepare an appropriate amount of assay buffer as described in Section 6.A.
2. To a sterile clear V-bottom 96-well plate, add 300μl of reference antibody starting dilution (dilu1, 2X final concentration) to wells A11 and B11 (see Figure 8).
3. Add 300μl of test antibodies 1 and 2 starting dilution (dilu1, 2X final concentration) to wells E11 and G11, respectively (see Figure 8).
4. Add 150μl of assay buffer to other wells in these four rows, from column 10 to column 2.
5. Transfer 150μl of the antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
6. Repeat equivalent twofold serial dilutions across the columns from right to left through column 3. Do not dilute into column 2.

Note: Wells A2, B2, E2 and G2 contain 150μl of 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 CD28 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.

6.E. Preparing CD28 Effector Cells

While maintaining the CD28 Effector Cells (TCR/CD3 (IL-2)), 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 as described in Section 4.B.
2. Count the CD28 Effector Cells by Trypan blue staining, and calculate the cell density and viability.
3. Transfer an appropriate amount of CD28 Effector Cells from the culture vessel to a 50ml conical tube or larger-sized centrifuge tube.
4. Pellet the cells at $130 \times g$ 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 2.5×10^6 cells/ml.
5. Count the cells again and adjust the volume of assay buffer to achieve a final cell density of 2.5×10^6 cells/ml. You will need at least 6ml of CD28 Effector Cells to fill 120 assay wells, or the inner 60 wells of two assay plates.

6.F. Adding CD28 Effector Cells and Antibody to Assay Plates

1. Take the 96-well assay plates containing FcγRIIb aAPC/CHO-K1 Cells out of 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, add 40μl of the appropriate antibody dilution (Figure 8) to the assay plates according to the plate layout in Figure 7.
3. Mix the CD28 Effector Cells by tube inversion and transfer the suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 40μl 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.
4. Add 80μl of assay buffer to each of the outside wells of the assay plates.
5. Cover the assay plates with lids and incubate in a 37°C, 5% CO₂ incubator for 5 hours.

Note: The 5-hour assay time was optimized using the Control Ab, Anti-CD28. We recommend optimizing assay time (5–24 hours) with your own antibody or other biologic samples.

6.G. Adding Bio-Glo™ Reagent

Note: Bio-Glo™ Reagent should be at ambient temperature (22–25°C) when added to assay plates.

1. After the 5-hour induction time, remove the assay plates from the incubator and equilibrate to ambient temperature for 15 minutes.
2. Using a manual multichannel pipette, add 80μl of Bio-Glo™ Reagent to the inner 60 wells of the assay plates, taking care not to create bubbles.
3. Add 80μl of Bio-Glo™ Reagent to wells B1, D1 and F1 of each assay plate to measure the background signal.
4. Incubate at ambient temperature for 5–10 minutes.



Note: Varying the incubation time will affect the raw RLU values but should not significantly change the EC₅₀ value and fold induction.

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

6.H. Data Analysis

1. Determine the plate background by calculating the average RLU from wells B1, D1 and F1.
2. Calculate fold induction =
$$\frac{\text{RLU (induced - background)}}{\text{RLU (no antibody control - background)}}$$
3. Graph data as RLU versus Log₁₀ [antibody] and fold induction versus Log₁₀ [antibody]. Fit curves and determine the EC₅₀ value of antibody response using appropriate curve fitting software (such as GraphPad Prism® software).

7. 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)	Choose an instrument designed for plate-reading luminescence detection. Instruments designed primarily for fluorescence detection are not recommended. Luminometers measure and report luminescence as relative values, and actual RLU numbers will vary between instruments. 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 leads to low RLU. Store and handle the Bio-Glo™ Reagent according to the instructions.
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 EC₅₀ value obtained in the CD28 Bioassay may vary from the EC₅₀ value obtained using other methods such as primary T cell-based assays. Optimize the assay incubation time within a range of 5–24 hours. If performing the assay for the first time, we recommend that you try using the FcγRIIb aAPC/CHO-K1 Cells since your antibody of interest may be dependent on crosslinking by FcγRIIb. 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 the plate reader background RLU, subtract the 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 may cause low assay performance and high assay variation. Avoid one-day cell passages whenever possible, especially with the CD28 Effector Cells. 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.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Variability in assay performance	Inappropriate cell handling during cell harvest, including long centrifuge times and high centrifuge speeds may cause low assay performance and high assay variation. Centrifuge the cells exactly according to the instructions. Inappropriate cell freezing/DMSO exposure may cause low assay performance and high assay variation. Freeze the cells exactly according to the instructions. Inappropriate cell counting methods may lead to variation in cell numbers in culture and assays and cause high assay variation. Ensure consistent and accurate cell counting methods.

8. References

- Rudd, C.E., Taylor, A. and Scheider, H. (2009) CD28 and CTLA-4 coreceptor expression and signal transduction. *Immunol. Rev.* **229**, 12–26.
- Thompson, C.B. *et al.* (1989) CD28 activation pathway regulates the production of multiple T-cell-derived lymphokines/cytokines. *Proc. Natl. Acad. Sci. USA.* **86**, 1333–7.
- Suntharalingam, G. *et al.* (2006) Cytokine storm in a phase I trial of the anti-CD28 monoclonal antibody TGN1412. *N. Engl. J. Med.* **355**, 1018–28.
- Wang, X. and Rivière, I. (2015) Manufacture of tumor- and virus-specific T lymphocytes for adoptive cell therapies. *Cancer Gene Ther.* **22**, 85–94.
- Wang, X. and Rivière, I. (2016) Clinical manufacturing of CAR T cells: Foundation of a promising therapy. *Mol. Ther. Oncolytics* **3**, 16015
- Grailer, J. *et al.* (2019) Quantitative cell-based bioassays to advance immunotherapy programs targeting immune checkpoint receptors. *Cancer Res.* **79** (13 Suppl.), Abstract no. 4113.
- Lubrano, E., Scriffignano, S. and Perrotta, F.M. (2018) Abatacept for the treatment of psoriatic arthritis. *Expert. Rev. Clin. Immunol.* **14**, 899–905.
- Vincenti, F. *et al.* (2016) Belatacept and long-term outcomes in kidney transplantation. *N. Engl. J. Med.* **374**, 333–43.

9. Appendix

9.A. Representative Assay Results

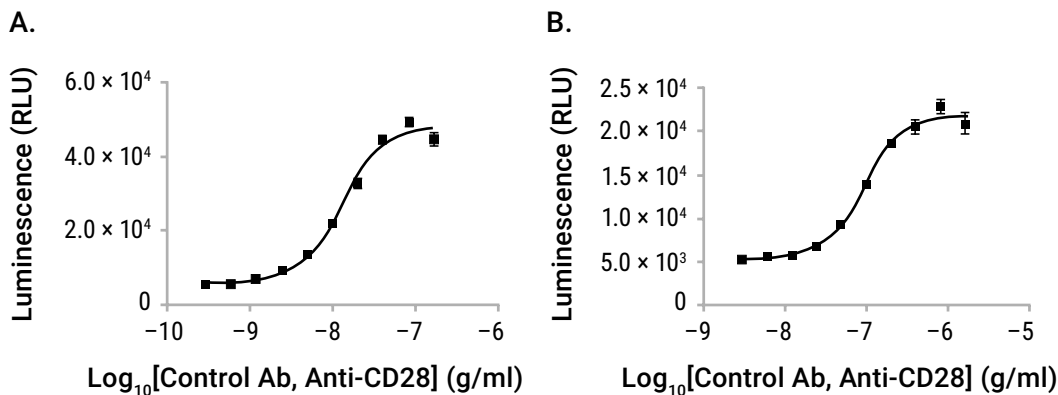


Figure 9. The CD28 Bioassay measures the activity of Control Ab, Anti-CD28. Panel A. FcγRIIb aAPC/CHO-K1 Cells were added to a 96-well assay plate 18 hours prior to the assay. On the day of the assay, a titration of Control Ab, Anti-CD28 (Cat.# K1231), and CD28 Effector Cells were added. After a 5-hour 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 EC₅₀ was 12ng/ml and the fold induction was 9.6. **Panel B.** aAPC/CHO-K1 Cells were added to a 96-well assay plate 18 hours prior to the assay. On the day of the assay, a titration of Control Ab, Anti-CD28 (Cat.# K1231), and CD28 Effector Cells were added. After a 5-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 EC₅₀ was 90ng/ml and the fold induction was 4.0.

9.B. Composition of Buffers and Solutions

Initial Cell Culture Medium for CD28 Effector Cells

- 90% RPMI 1640 with L-glutamine and HEPES
- 10% FBS

Cell Growth Medium for CD28 Effector Cells

- 90% RPMI 1640 with L-glutamine and HEPES
- 10% FBS
- 200µg/ml hygromycin B
- 1mm sodium pyruvate
- 0.1mm MEM nonessential amino acids

Cell Freezing Medium for CD28 Effector Cells

- 85% RPMI 1640 with L-glutamine and HEPES
- 10% FBS
- 5% DMSO

Assay Buffer

- 90% RPMI 1640 with L-glutamine and HEPES
- 10% FBS

Initial Cell Culture Medium for FcγRIIb aAPC/CHO-K1 Cells and aAPC/CHO-K1 Cells

- 90% Ham's F-12
- 10% FBS

Cell Growth Medium for FcγRIIb aAPC/CHO-K1 Cells

- 90% Ham's F-12
- 10% FBS
- 400µg/ml hygromycin B
- 10µg/ml Blasticidin

Cell Growth Medium for aAPC/CHO-K1 Cells

- 90% Ham's F-12
- 10% FBS
- 200µg/ml hygromycin B

Cell Plating Medium for FcγRIIb aAPC/CHO-K1 Cells, Propagation Model and aAPC/CHO-K1 Cells, Propagation Model

- 90% Ham's F-12
- 10% FBS

10. Summary of Changes

The following changes were made to the 6/26 revision of this document:

1. Removed Section 9.C, Related Products.
2. Made minor text and formatting edits.

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

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

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

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

^(c)U.S. Pat. No. 8,008,006.

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

©2021–2026 Promega Corporation. All Rights Reserved.

GloMax and Lumit are registered trademarks of Promega Corporation. Bio-Glo and Bio-Glo-NL are trademarks of Promega Corporation.

Corning and Costar are registered trademarks of Corning, Inc. GIBCO is a registered trademark of Life Technologies Corporation. GraphPad Prism is a registered trademark of GraphPad Software, Inc. JMP is a registered trademark of SAS Institute, Inc. Mr. Frosty is a registered trademark of Nalge Nunc International Corporation. Styrofoam is a registered trademark of Dow Chemical Company.

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

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

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