TECHNICAL MANUAL

TIGIT/CD155 Blockade Bioassay

Instructions for use of Products
J2201 and J2205

Revised 5/26
TM536

TIGIT/CD155 Blockade Bioassay

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

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1. Description

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. Inhibitory immune checkpoint receptors have been shown to perform critical roles in the maintenance of immune homeostasis but also have a significant role in cancer progression and autoimmune disease. Several immune checkpoint receptors such as Programmed Cell Death protein 1 (PD-1), Cytotoxic T-Lymphocyte Associated protein 4 (CTLA-4), T Cell immunoreceptor with immunoglobulin and Immunoreceptor Tyrosine-based Inhibitory Motif (TIGIT), and Lymphocyte Activation Gene-3 (LAG-3) have been identified. Blocking these receptors with monoclonal antibodies has proven to be an effective strategy to enhance anti-tumor immune responses and promote immune-mediated tumor rejection (1,2).

1. Description (continued)

TIGIT, also known as WUCAM and Vstm3, is an immune checkpoint protein expressed on lymphocytes. Highest expression levels are observed on effector $CD4^+$ and $CD8^+$ T cells, regulatory T cells, and NK cells (3). TIGIT has several distinct mechanisms of action that inhibit lymphocyte activation. First, it is an inhibitory counterpart of the co-stimulatory receptor CD226. When TIGIT is present on the surface of lymphocytes, it binds with much higher affinity than CD226 to their common ligand, CD155 (poliovirus receptor, PVR; 3). Therefore, TIGIT will outcompete CD226 for CD155 binding and thus negate CD226 co-stimulation. Second, TIGIT inhibits CD226 homodimerization in *cis*, preventing CD226 signaling (4). Third, the cytoplasmic tail of TIGIT contains an immunoreceptor tyrosine-based inhibitory motif (ITIM), which could potentially lead to inhibitory signaling. However, there is limited evidence to suggest that this is a major mechanism of TIGIT-induced inhibition in human T cells (5).

Current methods used to measure the activity of biologic drugs targeting TIGIT rely on primary human T cells and measurement of functional endpoints such as cell proliferation, cell surface marker expression, and interferon gamma (IFNγ) and interleukin-2 (IL-2) production. These assays are laborious and highly variable due to their reliance on donor primary cells, complex assay protocols and unqualified assay reagents. As a result, these assays are difficult to establish in a drug-development setting.

The TIGIT/CD155 Blockade Bioassay[a–e] (Cat.# J2201, J2205) is a bioluminescent cell-based assay that overcomes the limitations of existing assays and can be used to measure the potency and stability of antibodies and other biologics targeting TIGIT (6,7). The assay consists of two genetically engineered cell lines:

- **TIGIT Effector Cells:** Jurkat T cells engineered to express human TIGIT with a luciferase reporter driven by a native promoter that can respond to both TCR activation and CD226 co-stimulation
- **CD155 aAPC/CHO-K1 Cells:** CHO-K1 cells engineered to express human CD155 and an engineered cell-surface protein designed to activate the T cell receptor (TCR) complex in an antigen-independent manner.

The TIGIT Effector Cells and CD155 aAPC/CHO-K1 Cells are provided in thaw-and-use format as cryopreserved cells that can be thawed, plated and used in an assay without the need for cell culture and propagation.

When the two cell types are co-cultured, TIGIT inhibits CD226 activation and promoter-mediated luminescence. Addition of an anti-TIGIT antibody blocks the interaction of TIGIT with CD155 or inhibits the ability of TIGIT to prevent CD226 homodimerization, resulting in promoter-mediated luminescence (Figure 1). The TIGIT/CD155 Blockade Bioassay includes the necessary medium and serum to thaw, plate and assay the cells. The bioluminescent signal is quantified using the Bio-Glo™ Luciferase Assay System and a standard luminometer such as the GloMax® Discover System .

In addition to the TIGIT/CD155 Blockade Bioassay, we offer TIGIT Negative Cells (Cat.# J1921) for use as a negative control in the TIGIT/CD155 Blockade Bioassay. When co-cultured with CD155 aAPC/CHO-K1 Cells, the CD226 signaling in the TIGIT Negative Cells is activated, and this response is not affected by anti-TIGIT antibodies. We also offer Control Ab, Anti-TIGIT (Cat.# J2051), a blocking antibody, for use as a positive control.

The TIGIT Blockade Bioassay combines 1) a simple, add-mix-read two-day workflow with 2) TIGIT Effector Cells and CD155 aAPC/CHO-K1 Cells provided in a frozen, thaw-and-use format, and 3) an optimized protocol, which together yield a quantitative bioassay that exhibits low variability and high accuracy. The thaw-and-use cells provided in the TIGIT/CD155 Blockade Bioassay kits are manufactured under stringent quality control to provide high assay reproducibility with the convenience of an assay reagent that eliminates the need for continuous cell propagation.

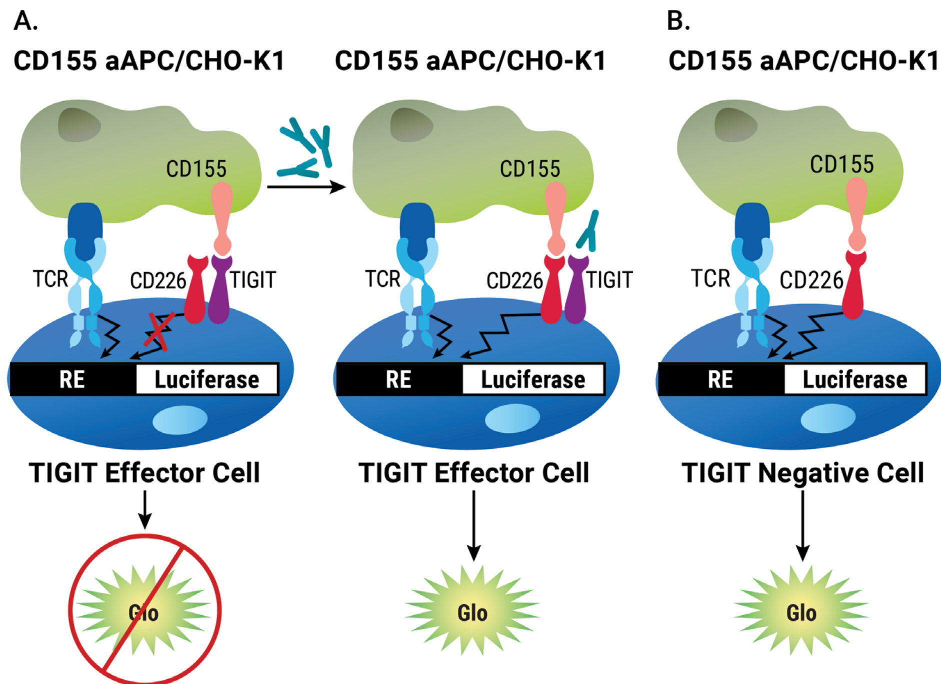

Figure 1. Representation of the TIGIT/CD155 Blockade Bioassay. The bioassay consists of two genetically engineered cell lines, TIGIT Effector Cells and CD155 aAPC/CHO-K1 Cells. **Panel A.** When co-cultured, TIGIT inhibits CD226 pathway-activated luminescence. The addition of anti-TIGIT antibody blocks the TIGIT/CD155 interaction, thereby re-establishing CD226 pathway-activated luminescence, which can be detected in a dose-dependent manner by addition of Bio-Glo™ Reagent and quantitation with a luminometer. **Panel B.** When co-cultured with non-TIGIT-expressing Effector Cells (Cat.# J1921), CD155 induces luminescence by activation of the CD226 pathway.

1. Description (continued)

The TIGIT/CD155 Blockade Bioassay reflects the mechanism of action (MOA) of biologics designed to block the TIGIT/CD155 interaction. Specifically, CD226-mediated luminescence is detected following the addition of anti-TIGIT blocking antibodies but not following addition of anti-PD-1, anti-PD-L1 or anti-CTLA-4 blocking antibodies (Figure 2). The bioassay is prequalified according to 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 assay can be performed in a two-day time frame. The bioassay workflow is simple and robust, and compatible with both 96-well and 384-well plate formats used for antibody screening in early drug discovery (Figure 4). In addition, the bioassay can be used with up to 100% human serum (in antibody samples) with minimal impact on fold induction (Figure 5), indicating potential for further development into a neutralizing antibody bioassay.

It is increasingly common during drug development to analyze potential therapeutic antibodies for Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC activity). Another application of the TIGIT/CD155 Blockade Bioassay is the ability to measure ADCC activity of anti-TIGIT-blocking antibodies by combining ADCC Reporter Bioassay Jurkat Effector cells, available separately (Cat.# G7010), with TIGIT Effector Cells (Figure 6).

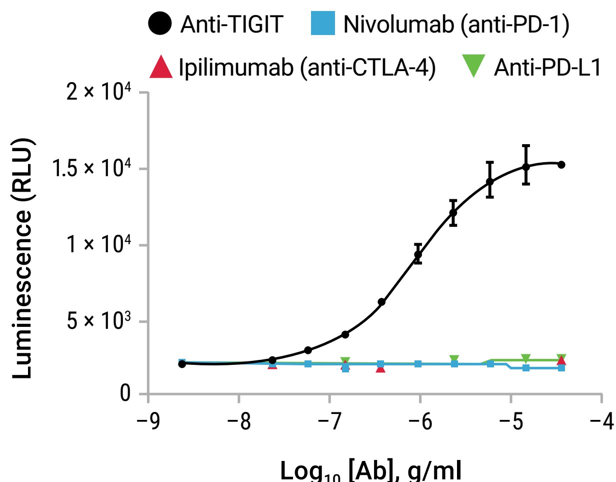

Figure 2. The TIGIT/CD155 Blockade Bioassay reflects the mechanism of action (MOA) and specificity of biologics designed to block the TIGIT/CD155 interaction. TIGIT Effector Cells were incubated with CD155 aAPC/CHO-K1 Cells in the presence of a serial titration of Control Ab, Anti-TIGIT (Cat.# J2051), anti-PD-1 (nivolumab), anti-CTLA-4 (ipilimumab) or anti-PD-L1-blocking antibodies as indicated. 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 TIGIT/CD155 Blockade Bioassay Shows Precision, Accuracy and Linearity.

Parameter	Results	
Accuracy	% Expected Relative Potency	% Recovery
	50	101.8
	70	102.8
	140	101.0
	200	98.5
Repeatability (% CV)	100% (Reference)	11.0
Intermediate Precision (% CV)		9.7
Linearity (r^2)		0.999
Linearity (y = mx + b)		y = 0.974x + 3.33

A 50–200% theoretical potency series of Control Ab, Anti-TIGIT, was analyzed in triplicate in three independent experiments performed on three days by two analysts. 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.

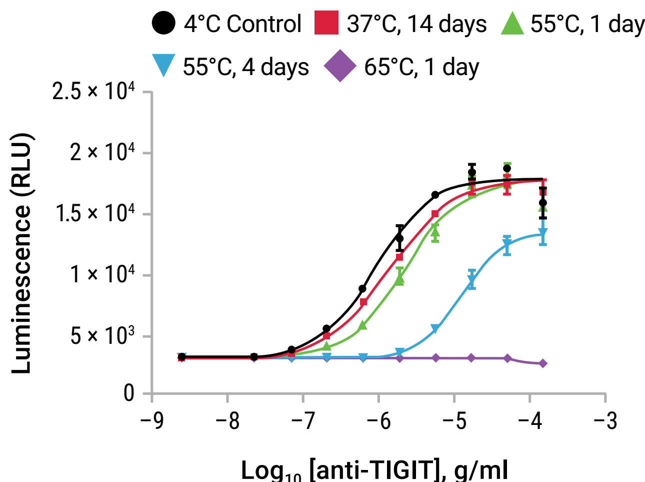

Figure 3. The TIGIT/CD155 Blockade Bioassay is stability-indicating. Samples of Control Ab, Anti-TIGIT, were maintained at 4°C (control) or heat-treated at the indicated temperatures and times, and then analyzed using the TIGIT/CD155 Blockade Bioassay. Bio-Glo™ Reagent was added and luminescence quantified 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.

1. Description (continued)

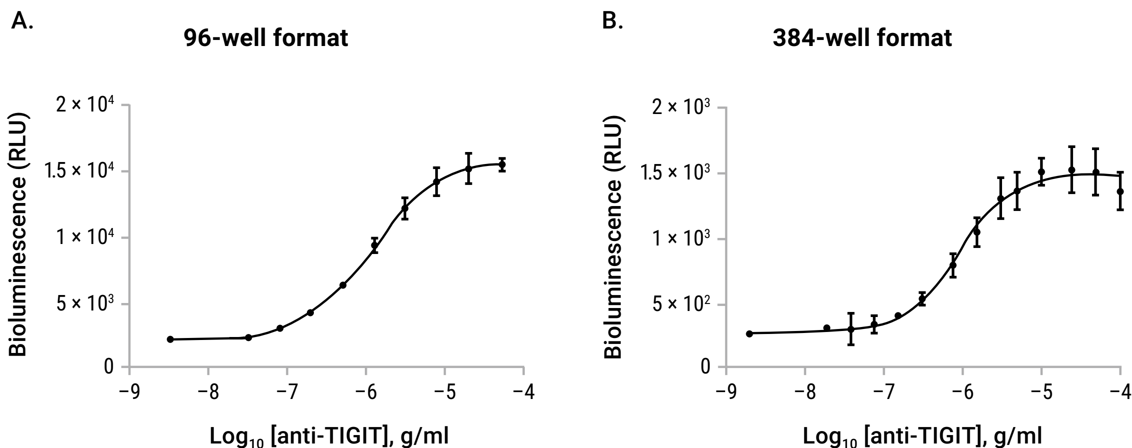

Figure 4. The assay is amenable to 384-well plate format and compatible with laboratory automation. Panel A. The TIGIT/CD155 Blockade Bioassay was performed in 96-well plates as described in this technical manual using Control Ab, Anti-TIGIT. **Panel B.** The TIGIT/CD155 Blockade Bioassay was performed in 384-well format using a Multidrop™ Combi nL (ThermoFisher) dispenser. TIGIT Effector Cells were plated at 6×10^4 cells in 10µl/well. Next, Control Ab, Anti-TIGIT, was serially diluted and added to the plate at 5µl/well. Finally, CD155 aAPC/CHO-K1 cells were added at 1.5×10^4 cells in 5µl/well. After a 6-hour incubation, 20µl 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 EC_{50} values were 1.1µg/ml and 0.9µg/ml, and the fold inductions were 8.0 and 6.1 for 96- and 384-well format, respectively. Data were generated using thaw-and-use cells.

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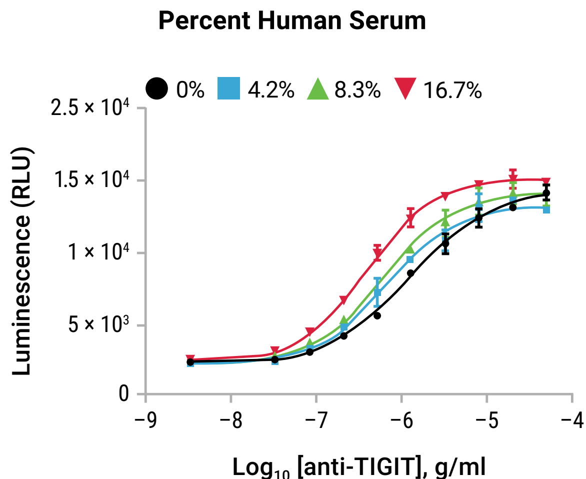

Figure 5. The TIGIT/CD155 Blockade Bioassay is tolerant to human serum. Control Ab, Anti-TIGIT, was analyzed in the absence or presence of increasing concentrations of pooled normal human serum (0–100% in the antibody sample), resulting in final assay concentration of human serum (0–16.7%). Bio-Glo™ 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 TIGIT blockade assay is tolerant to serum with this human serum pool. A different human serum pool showed similar effects on the assay (data not shown).

1. Description (continued)

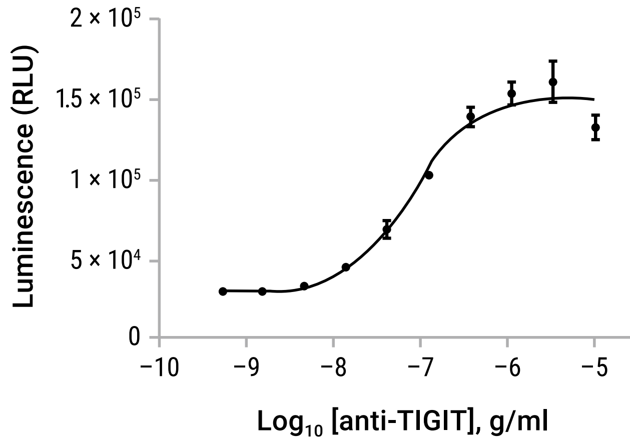

Figure 6. The TIGIT/CD155 Blockade Bioassay can be used to measure ADCC activity. A 1:1 ratio of TIGIT Effector Cells (used as target cells in this application) and ADCC Effector Jurkat cells, available separately (Cat.# G7010), were incubated for 6 hours in the presence of a research-grade anti-TIGIT antibody (human IgG1 isotype). 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.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
TIGIT/CD155 Blockade Bioassay	**1 each**	**J2201**

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

- 1 vial TIGIT Effector Cells (0.5ml)
- 1 vial CD155 aAPC/CHO-K1 cells (0.5ml)
- 36ml RPMI 1640 Medium
- 4ml Fetal Bovine Serum
- 2 vials Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 2 × 10ml Bio-Glo™ Luciferase Assay Buffer

PRODUCT	SIZE	CAT.#
TIGIT/CD155 Blockade Bioassay 5X	**5 each**	**J2205**

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

- 5 vials TIGIT Effector Cells (0.5ml)
- 5 vials CD155 aAPC/CHO-K1 cells (0.5ml)
- 5 × 36ml RPMI 1640 Medium
- 5 × 4ml Fetal Bovine Serum
- 10 vials Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 10 × 10ml Bio-Glo™ Luciferase Assay Buffer

Note: The TIGIT/CD155 Blockade Bioassay components are shipped separately because of differing temperature requirements. The TIGIT Effector Cells and CD155 aAPC/CHO-K1 Cells are shipped on dry ice. The Bio-Glo™ Luciferase Assay Substrate and Buffer and Fetal Bovine Serum are shipped on dry ice, separately from the cells. The RPMI 1640 Medium is shipped at ambient temperature.

Storage Conditions:

- Upon arrival, immediately transfer the cell vials to below –140°C (freezer or liquid nitrogen vapor phase) for long-term storage. Do not store cell vials submerged in liquid nitrogen. **Do not** store cell vials at –80°C because this will decrease cell viability and cell performance.
- Store Bio-Glo™ Luciferase Assay Substrate, Bio-Glo™ Luciferase Assay Buffer and Fetal Bovine Serum at –30°C to –10°C. Avoid multiple freeze-thaw cycles of the serum.
- For optimal performance, use reconstituted Bio-Glo™ Reagent on the day of preparation. However, once reconstituted, Bio-Glo™ Reagent can be stored at –30°C to –10°C for up to 6 weeks.
- Store RPMI 1640 Medium at +2°C to +10°C protected from fluorescent light.

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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.

The TIGIT/CD155 Blockade Bioassay is intended to be used with user-provided antibodies or other biologics designed to block the interaction of TIGIT with its ligand, CD155. Control Ab, Anti-TIGIT (Cat.# J2051), and TIGIT Negative Cells (Cat.# J1921) are available separately for use in assay optimization and routine quality control. We strongly recommend including Control Ab, Anti-TIGIT, as a positive control in the first few assays to gain familiarity with the assay. Data generated using these reagents are shown in Figures 2–5 and Section 7.A, Representative Assay Results.

The TIGIT Effector Cells and CD155 aAPC/CHO-K1 Cells are provided in frozen, thaw-and-use format and are ready to be used without any additional cell culture or propagation. When thawed and diluted as instructed, the cells will be at the appropriate concentration for the assay. The cells are sensitive, and care should be taken to follow cell thawing and plating procedures as described.

The TIGIT/CD155 Blockade Bioassay produces a bioluminescent signal and requires a sensitive luminometer or luminescence plate reader for the detection of luminescence. 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. Relative luminescence unit 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 robustness of the raw data, but should not affect the measured relative potency of test samples.

3.A. Materials to Be Supplied by the User

- user-defined anti-TIGIT blocking antibodies or other biologics
- solid-white, flat-bottom 96-well assay plates (e.g., Corning® Cat.# 3917)
- sterile, clear V-bottom 96-well plate with lid (e.g., Costar® Cat.# 3896 or Linbro Cat.# 76-223-05) for preparing antibody dilutions
- pipettes (single-channel and 12-channel); for best results use both manual and electronic pipettes as needed
- sterile 15ml and 50ml conical tubes
- sterile reagent reservoirs (e.g., Corning® Cat.# 4870)
- 37°C, 5% CO_2 incubator
- 37°C water bath
- plate reader with glow luminescence measuring capability or luminometer (e.g., GloMax® Discover System or equivalent)

4. Assay Protocol

This assay protocol illustrates the use of the TIGIT/CD155 Blockade Bioassay to test two antibody samples against a reference sample in a single assay run. Each test and reference antibody is run in triplicate, in a ten-point dilution series, in a single 96-well assay plate using the inner 60 wells. Other experimental and plate layouts are possible but may require further optimization.

When preparing test and reference antibodies, choose an appropriate starting concentration and dilution scheme to achieve a full dose-response curve with proper upper and lower asymptotes and sufficient points on the slope. For reference, we use 50µg/ml as a starting concentration (1X) and 2.5-fold dilution when testing Control Ab, Anti-TIGIT, to achieve full dose curves.

Note: The order of addition of Effector and Target cells is different from the PD-1 Blockade Bioassay and PD-1+TIGIT Combination Bioassay. Please read this protocol carefully before you begin.

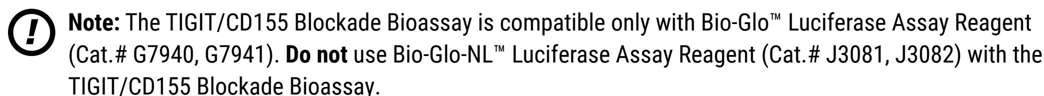
4.A. Preparing Assay Buffer, Bio-Glo™ Reagent and Antibody Samples

1. **Assay Buffer:** On the day before performing the assay, prepare 35ml of assay buffer (90% RPMI 1640/10% FBS) in a conical tube. Thaw the FBS overnight at 4°C or in a 37°C water bath on the day of use. Add 3.5ml of FBS to 31.5ml of RPMI 1640 Medium to yield 90% RPMI 1640/10% FBS. Mix well and warm to 37°C before use.

   You will use 12ml assay buffer the day before the assay. Store the remaining assay buffer at 4°C overnight. The day of the assay, warm the remaining assay buffer to 37°C before use.

   Note: The recommended assay buffer contains 10% FBS. This concentration of FBS works well for the Control Ab, Anti-TIGIT, 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%.

2. **Bio-Glo™ Reagent:** For reference, 2 × 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. When stored at ambient temperature, Bio-Glo™ Reagent will lose 18% activity after 24 hours.



Note: The TIGIT/CD155 Blockade Bioassay is compatible only with Bio-Glo™ Luciferase Assay Reagent (Cat.# G7940, G7941). **Do not** use Bio-Glo-NL™ Luciferase Assay Reagent (Cat.# J3081, J3082) with the TIGIT/CD155 Blockade Bioassay.

3. **Test and Reference Samples:** Using assay buffer as the diluent, prepare starting dilutions (dilu1, 6X final concentration) of two test antibodies (150µl each) and one reference antibody (300µl) in 1.5ml tubes. Store the tubes containing antibody starting dilutions appropriately before making antibody serial dilutions.

   Note: If you are using Control Ab, Anti-TIGIT, as a reference antibody in your assay, prepare 300µl of 300µg/ml starting dilution (dilu1, 6X final concentration) by adding 90µl of Control Ab, Anti-TIGIT, stock (1mg/ml) to 210µl of assay buffer. Store the antibody starting dilution on ice until ready to use in the assay.

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4. Assay Protocol (continued)

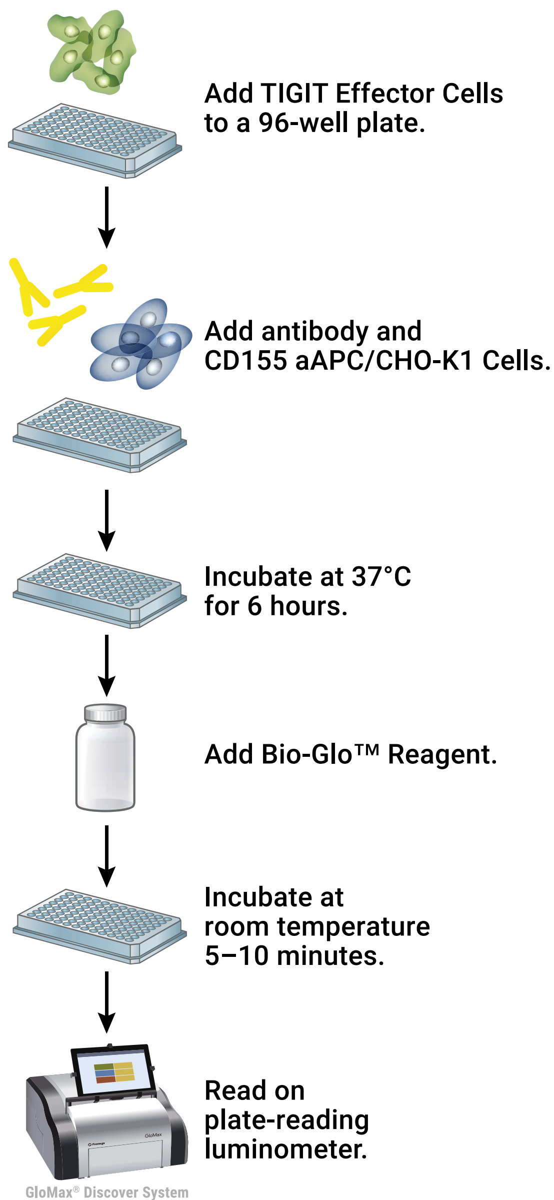

Figure 7. Schematic protocol for the TIGIT/CD155 Blockade Bioassay.

4.B. Plate Layout Design

For the protocol described here, use the plate layout illustrated in Figure 8 as a guide. The protocol describes serial replicate dilutions (n = 3) of test and reference antibodies 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	Test Ab
E	B	no Ab	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Reference 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 8. Example plate layout showing non-clustered sample locations of test and reference antibody dilution series and wells containing Assay Buffer (denoted by "B") alone.

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4.C. Preparing TIGIT Effector Cells

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

The thaw-and-use TIGIT Effector Cells included in this kit are sensitive, and care should be taken to follow the cell thawing and plating procedures exactly as described. Do not overmix or overwarm the cell reagents. No additional cell culture or cell manipulation is required or recommended. We recommend that you thaw and dilute a maximum of two vials of thaw-and-use cells at one time.

1. On the day before performing the assay, prepare 35ml of assay buffer as described in Section 4.A.
2. Transfer 12ml of assay buffer to a 15ml conical tube. Warm in a 37°C water bath. Store remaining assay buffer at 4°C overnight.
3. Remove one vial of TIGIT Effector cells from storage at –140°C and transfer to the bench on dry ice. Warm the cells in a 37°C water bath until just thawed (about 2–3 minutes). While thawing, gently agitate and visually inspect.
4. Gently mix the cell suspension by pipetting, and then transfer the cells (0.5ml) to the 15ml conical tube containing 12ml of prewarmed (37°C) assay buffer. Mix well by gently inverting.
5. Transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 80µl of the cell suspension to each of the inner 60 wells of two 96-well, white, flat-bottom assay plates.
6. Add 120µl of prewarmed (37°C) Assay Buffer to each of the outside wells of the assay plates.
7. Cover the assay plates with a lid and incubate the cells overnight (16–20 hours) in a 37°C, 5% CO_2 incubator.

4.D. Preparing and Adding Antibody Serial Dilutions

The instructions described here are for preparation of a single stock of 2.5-fold serial dilutions of a single antibody for analysis in triplicate (90µl of each antibody 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 2.5-fold serial dilutions, you will need 300µl of reference antibody at 6X the highest antibody concentration in your dose-response curve. You will need 150µl of each test antibody at 6X 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-TIGIT, as a control in the assay, follow the instructions below to prepare 2.5-fold serial dilutions.

1. Remove the assay buffer that was made the day before from 4°C storage and warm in a 37°C water bath.
2. Transfer 3ml of assay buffer into a fresh 15ml conical tube. Set tube aside to be used in Section 4.E.
3. Make antibody starting dilutions as described using assay buffer as the diluent.
4. To a sterile, clear, V-bottom 96-well plate, add 150µl of reference antibody starting dilution (dilu1, 6X final concentration) to wells A11 and B11 (see Figure 9).
5. Add 150µl of test antibodies 1 and 2 starting dilution (dilu1, 6X final concentration) to wells E11 and G11, respectively (see Figure 9).

6. Add 90µl of assay buffer to the other wells in these four rows, from column 10 to column 2.
7. Transfer 60µl of the antibody starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
8. Repeat equivalent 2.5-fold 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 90µl of assay buffer without antibody as a negative control.
9. Remove the lid from the 96-well assay plates containing TIGIT Effector Cells.
10. Using an electronic multichannel pipette, add 20µl of the appropriate antibody dilution (see Figure 9) to the pre-plated TIGIT Effector Cells according to the plate layout in Figure 8.
11. Cover the assay plates with a lid and keep at ambient temperature (22–25°C while preparing the CD155 aAPC/CHO-K1 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 9. Example plate layout showing antibody serial dilutions.

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4.E. Preparing and Plating CD155 aAPC/CHO-K1 Cells

Note: The thaw-and-use CD155 aAPC/CHO-K1 cells included in this kit are sensitive, and care should be taken to follow the cell thawing and plating procedures exactly as described. Do not overmix or overwarm the cell reagents. No additional cell culture or manipulation is required or recommended. We recommend that you thaw and dilute a maximum of two vials of thaw-and-use cells at any one time.

1. Remove one vial of CD155 aAPC/CHO-K1 Cells from storage at –140°C and transfer to the bench on dry ice. Thaw the cells in a 37°C water bath until just thawed (about 2–3 minutes). While thawing, gently agitate and visually inspect.
2. Gently mix the cell suspension by pipetting, and then transfer the cells (0.5ml) to the 15ml conical tube containing 3ml of assay buffer (from Step 4.D). Mix well by gently inverting or pipetting 1–2 times.
3. Transfer the cell suspension to a sterile reagent reservoir. Using a multichannel pipette, immediately dispense 20µl of the cell suspension to the pre-plated TIGIT Effector Cells and anti-TIGIT antibody. The final assay volume is 120µl.
4. Cover the assay plates with a lid and incubate for 6 hours in a 37°C, 5% CO_2 incubator.

4.F. Adding Bio-Glo™ Reagent

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

1. Remove the assay plates from the incubator and equilibrate to ambient temperature for 10–15 minutes.
2. Using a manual multichannel pipette, add 120µl of Bio-Glo™ Reagent to the inner 60 wells of the assay plates, taking care not to create bubbles.
3. Add 120µl of Bio-Glo™ Reagent to wells B1, C1 and D1 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 relative light unit (RLU) values but should not significantly change the EC_{50} value and fold induction.
5. Measure luminescence using a luminometer or luminescence plate reader.

4.G. Data Analysis

1. Determine the plate background by calculating the average RLU from wells B1, C1 and D1.
2. Calculate fold induction:

$$\text{Fold Induction} = \frac{RLU_{\text{Induced – background}}}{RLU_{\text{No antibody control – background}}}$$

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).

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	**Causes and Comments**
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 older models of luminometers with low sensitivity should be avoided. We recommend using a high gain setting if you are using a luminometer with an adjustable gain.
	Insufficient cells per well can lead to low RLU. Handle and plate the 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 full dose response with complete upper and lower asymptotes. The EC_{50} value obtained in the TIGIT/CD155 Blockade Bioassay may vary from the EC_{50} obtained using other methods such as primary T cell-based assays.
	If untreated control RLU is less than 100-fold above plate reader background RLU, subtract plate reader background RLU from all samples prior to calculating fold induction.

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6. References

1. Mahoney, K.M. *et al.* (2015) Combination cancer immunotherapy and new immunomodulatory targets. *Nature Rev. Drug Discov.* **14**, 561–84.
2. Melero, I. *et al.* (2015) Evolving synergistic combinations of targeted immunotherapies to combat cancer. *Nature Rev. Cancer* **15**, 457–72.
3. Yu, X. *et al.* (2009) The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nature Immunol.* **10**, 48–57.
4. Johnston, R.J. *et al.* (2014) The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell* **26**, 923–37.
5. Manieri, N.A. *et al.* (2017) TIGIT: A key inhibitor of the cancer immunity cycle. *Trends Immunol.* **38**, 20–8.
6. Grailer, J.J. *et al.* (2017) Quantitative cell-based bioassays to advance individual or combination immune checkpoint immunotherapy. *Cancer Res.* **77**, (13 suppl.), Abstract no. 5610.
7. Cheng, Z.-J. *et al.* (2016) Bioassays power combination immunotherapy. *Genetic Engineering & Biotechnology News* **36**, 9.

7. Appendix

7.A. Representative Assay Results

The following data were generated with the TIGIT/CD155 Blockade Bioassay using Control Ab, Anti-TIGIT, (Figure 10).

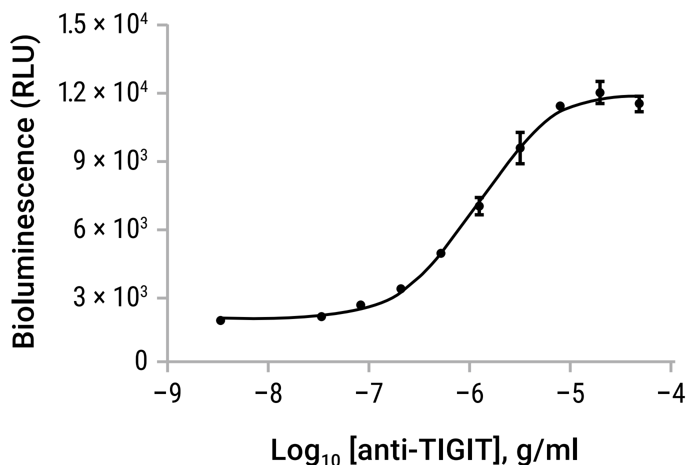

Figure 10. The TIGIT/CD155 Blockade Bioassay measures the activity of Control Ab, Anti-TIGIT. TIGIT Effector Cells were plated overnight. The following day, a titration of Control Ab, Anti-TIGIT, was added followed by CD155 aAPC/CHO-K1 Cells. After 6 hours, Bio-Glo™ Reagent was added and luminescence measured using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. The EC_{50} value was 1.2µg/ml, and the fold induction was 7.1. Data were generated using thaw-and-use cells.

8. Summary of Changes

The following changes were made to the 5/26 revision of this document:

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

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