Promega

TECHNICAL MANUAL

T Cell Activation Bioassay (TCRαβ-KO), Propagation Model

Instructions for Use of Products
GA1162, GA1172, GA1182, GA1210, GA1220 and GA1230

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T Cell Activation Bioassay (TCRαβ-KO), 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

Adoptive T cell immunotherapy is a cancer treatment strategy in which genetically modified T cells are made tumor specific via introduction of an antigen receptor, which improves their ability to recognize and destroy tumors. T cell receptor-engineered T cell therapy (TCR-T) and chimeric antigen receptor T cell therapy (CAR-T) are two types of adoptive T cell immunotherapy. CAR-T features engineered T cells expressing a CAR composed of antibody domains fused to T cell signaling domains. The antibody domains confer specificity to a tumor antigen, the binding of which will induce T cell activation. CAR-T has demonstrated clinical efficacy for hematologic malignancies, and several CAR-T products have been approved for clinical use (1).

TCR-T features engineered expression of a transgenic TCR by allogeneic or (typically) autologous T cells. TCRs have evolved to respond effectively to relatively low levels of antigens and TCRs can recognize almost any protein via the major histocompatibility complex (MHC) system, making TCRs exquisitely sensitive to altered self (tumor-associated) or pathogen-derived antigens.

The TCR complex consists of the TCR alpha (α) and beta (β) chains in association with accessory signaling molecules (e.g., CD3). Individual T cells typically express one alpha and one beta chain resulting from developmental somatic gene rearrangement of the TCR locus. Introduction of exogenous TCR alpha and beta chains result in the formation of mixed TCR complexes (e.g., alpha chain from endogenous TCR, beta chain from exogenous TCR; 2). By their nature, mixed αβ TCR pairs result in TCRs of altered antigen specificity. Furthermore, pairing of endogenous and exogenous TCR chains results in low expression of the "preferred" exogenous TCR pairing, and reduced recognition of the cognate antigen (3,4).

CD4 and CD8 are T cell coreceptors that bind to nonpolymorphic regions on MHC, where they enhance signaling and sensitivity of low affinity TCRs or TCRs responding to low levels of antigen. CD4, typically expressed on helper and regulatory T cells, binds MHC class II (MHCII), present on professional antigen presenting cells (APCs). In contrast, CD8 is typically expressed on cytotoxic T cells, and binds to MHCI, which is expressed by all somatic cells. MHCII typically presents extracellular (e.g., pathogen-derived) antigens, while MHCI presents intracellular (e.g., self (tumor) or viral) antigens.

Cell-based immunotherapies include adaptive T cell therapies that rely on modification of T cells with transgenic TCRs that are capable of binding to antigen on the target cells. Functional bioassays are needed in the development and utilization of transgenic TCRs. Current methods rely on binding affinity measurements of TCR-MHC/peptide, which are not necessarily indicative of the ability of a TCR to effectively activate T cells (5–7). Other methods rely on primary human T cells and measurement of functional endpoints, such as cell proliferation, activation-induced marker analysis and cytokine production. These assays are laborious and highly variable due to their reliance on donor T cells, complex assay protocols and unqualified assay reagents. Results are additionally confounded by the existing TCR endogenously expressed by primary T cells.

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The T Cell Activation Bioassay (TCRαβ-KO), Propagation Model[a–h] (Cat.# GA1162, GA1172, GA1182), is a bioluminescent reporter cell-based assay that overcomes the limitations of existing assays. It can be used to measure the potency of transgenic TCR constructs to activate T cells without the constraints of endogenous TCR expression (Figures 1 and 2). The assay consists of a genetically engineered Jurkat T cell line with endogenous TCR alpha and beta chains knocked out using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9. These cells also express a luciferase reporter driven by a TCR pathway-dependent promoter.

The TCRαβ-KO 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, this assay is available in a cell bank format (Cat.# GA1210, GA1220, GA1230), which includes vials of TCRαβ-KO cells for customers that are planning to expand the CPM vials. We offer separately a TCR Transfection Positive Control Kit that includes a plasmid encoding a Positive Control TCR, a cognate Positive Control Peptide and APCs in thaw-and-use format.

Introduction of TCR alpha and beta chains into the TCRαβ-KO Cells results in transgenic TCR expression on the cell surface (Figure 2). Activation of transgenic TCR-expressing TCRαβ-KO Cells by cognate peptide and MHC-expressing APCs results in potent TCR activation and promoter-mediated luminescence (Figure 2). CD8+ and CD4+ TCRαβ-KO Cells enable efficient analysis of MHCI- and MHCII-restricted peptides/TCRs (Figure 3). Additionally, the CD4+/CD8+ double-positive TCRαβ-KO Cells allow transgenic TCR screening without biasing for CD4- or CD8-dependence (Figure 3). The bioluminescent signal is quantified using the Bio-Glo-NL™ Luciferase Assay System, and a standard luminometer such as the GloMax® Discover System (see Related Products, Section 9.C).

The T Cell Activation Bioassay (TCRαβ-KO) can be used throughout the development of TCR-T. Both TCR affinity and surface expression level dictate the magnitude of T cell responses (6,7). TCR chain sequences can affect surface expression of TCR constructs (8). Therefore, it is important to test empirically the expression level of TCR constructs. TCR expression studies comparing the surface expression level of different TCR constructs can be performed using the TCRαβ-KO cells (Figure 4). The bioassay enables functional testing of transgenic TCRs for antigen ranking, specificity and safety testing (Figure 5). Additionally, TCR dependence on CD4 or CD8 co-expression can be interrogated during TCR screening and development (Figure 6).

In addition to the development of TCR-T, TCRαβ-KO Cells facilitate many applications for which detection of specific antigens is required. Transgenic TCR-expressing stable reporter cell lines can be developed using TCRαβ-KO Cells for long-term use in vaccine and immunotherapy development, including for quality control assays. We have developed a suite of stable transgenic TCR-expressing reporter cells derived from the TCRαβ-KO Cells that are specific for viral (e.g., CMV, HPV, influenza, etc.) or tumor (e.g., Melan-A (MART-1), etc.) antigens. For more information on existing available TCR-expressing cell lines and custom cell line development, visit **www.promega.com/support/**

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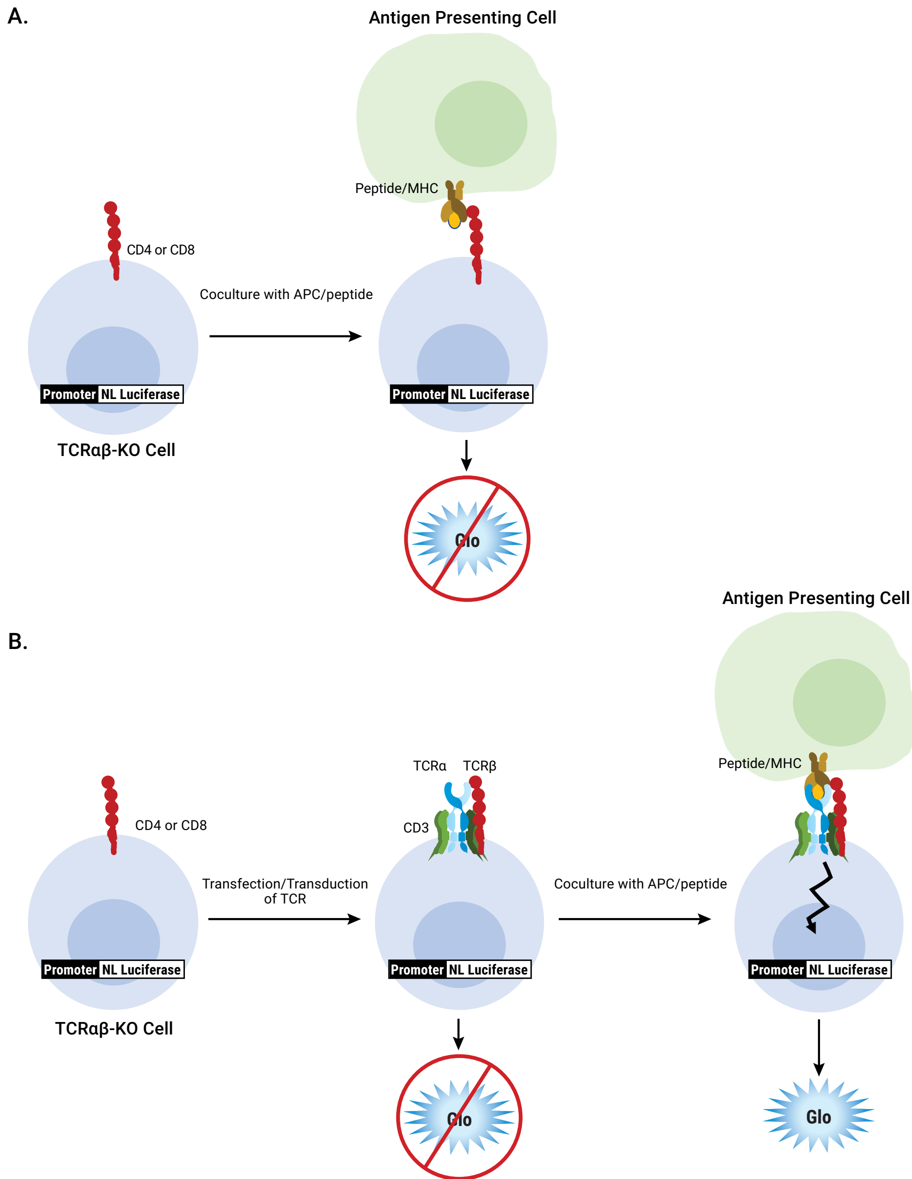

Figure 1. Representation of the T Cell Activation Bioassay (TCRαβ-KO). Panel A. Without expressing a transgenic TCR, TCRαβ-KO Cells are not activated by peptide or MHC, resulting in low light output. **Panel B.** TCRαβ-KO Cells transfected or transduced to express a TCR are activated by APCs and cognate antigen, inducing TCR pathway-activated luminescence.

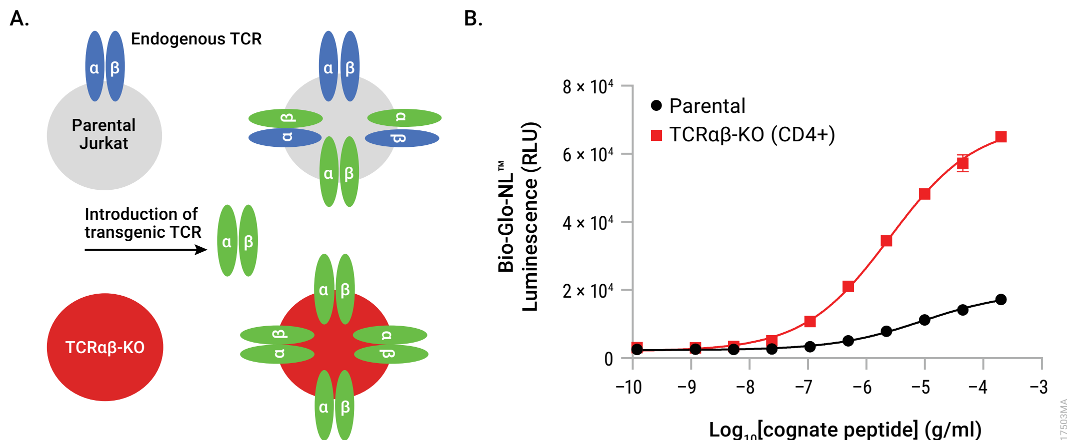

Figure 2. Comparison of TCRαβ-KO Cells and parental Jurkat cells (with endogenous TCR). Panel A. Schematic of introduction of transgenic TCR into parental Jurkat cells and TCRαβ-KO Cells. Introduction of transgenic TCR into parental Jurkat cells results in expression of mismatched TCR chains consisting of endogenous and transgenic TCRαβ chains. Transgenic TCR expression in TCRαβ-KO Cells results in a single species of TCRαβ pairing due to the lack of endogenous TCR. **Panel B.** Functional assay response of the cells described in **Panel A**. TCR-transfected parental Jurkat cells (endogenously CD4+) and TCRαβ-KO (CD4+) Cells were cocultured with HLA-DR-positive cells and a titration of cognate peptide. After six hours, Bio-Glo-NL™ 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 2.5µg/ml and 9.1µg/ml, respectively, and the fold inductions were 22 and 8 for TCRαβ-KO (CD4+) Cells and parental Jurkat cells, respectively.

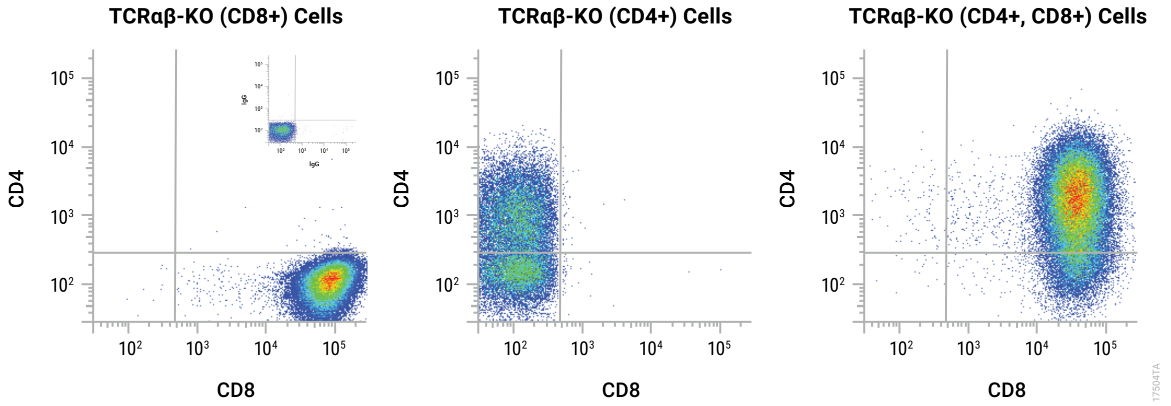

Figure 3. Expression of CD4 and CD8 on TCRαβ-KO (CD8+), TCRαβ-KO (CD4+) and TCRαβ-KO (CD4+, CD8+) Cells. TCRαβ-KO Cells were labeled with fluorochrome-conjugated antibodies binding to CD8 (x axis, Ab clone SK1) and CD4 (y axis, Ab clone OKT4) and analyzed on a BD LSRFortessa™ X-20 flow cytometer. Data analysis was performed with FlowJo™ software. The inset plot shows isotype-matched control antibodies.

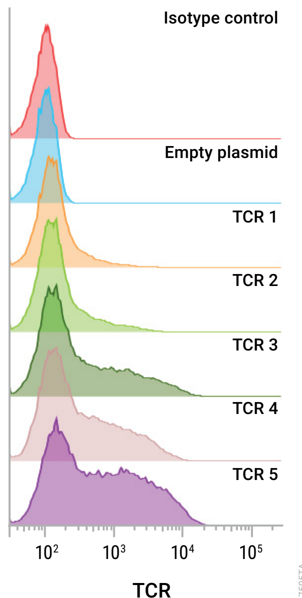

Figure 4. TCR expression in TCRαβ-KO Cells. TCRαβ-KO (CD8+) Cells were transiently transfected with empty plasmid or plasmids encoding five separate TCRs. After 48 hours, TCR surface expression was analyzed by flow cytometry (anti-TCR antibody clone IP26) on a BD LSRFortessa™ X-20 flow cytometer. Data analysis was performed with FlowJo™ software.

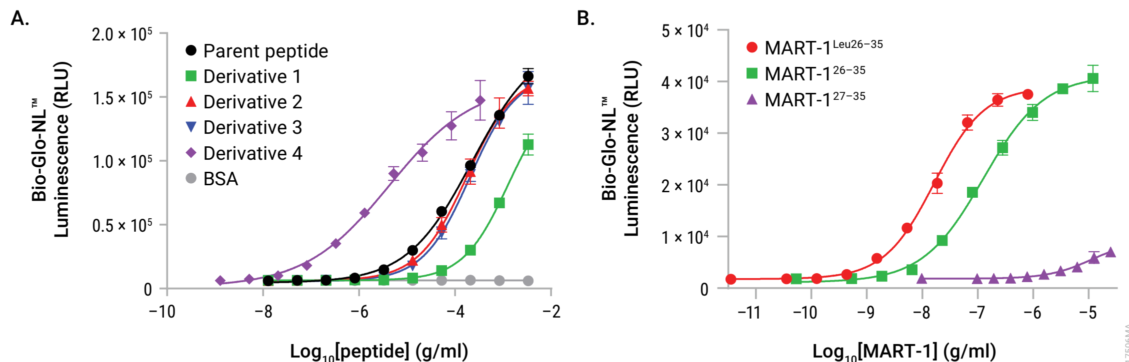

Figure 5. TCRαβ-KO Cells enable antigenic peptide potency ranking and safety profiling. Panel A. TCRαβ-KO (CD4+) Cells were transiently transfected with the HA1.7 ($HA^{307-319}$-specific) TCR. After 48 hours, transfected cells were cocultured with HLA-DR-positive cells and a titration of $HA^{307-319}$ (parent peptide) or derivative peptides with amino acid substitutions as shown. After 6 hours, Bio-Glo-NL™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to four-parameter logistic curves using GraphPad Prism® software. Derivatization of the $HA^{307-319}$ produced peptides that were more potent (derivative 4) and less potent (derivative 1) activators of the HA1.7 TCR. **Panel B.** TCRαβ-KO (CD8+) Cells were transiently transfected with the DMF5 (MART-1-specific) TCR. After 48 hours, transfected cells were cocultured with HLA-A2-positive cells and a titration of Melan-A (MART-1) peptides as shown. $MART\text{-}1^{Leu26-35}$ contains a leucine at position 2 of the native sequence. After 6 hours, Bio-Glo-NL™ 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 for $MART\text{-}1^{Leu26-35}$ and $MART\text{-}1^{26-35}$ were 16ng/ml and 130ng/ml, respectively.

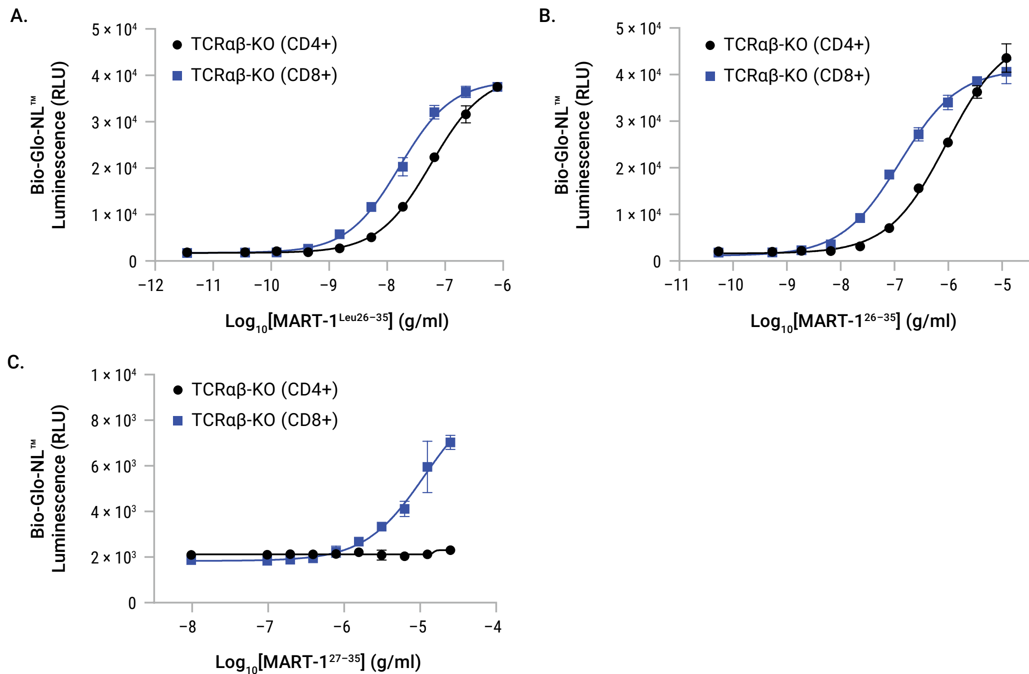

Figure 6. The DMF4 TCR exhibits CD8 dependence in TCRαβ-KO Cells. TCRαβ-KO (CD4+) Cells and TCRαβ-KO (CD8+) Cells were transiently transfected with the CD8-dependent TCR DMF4 (MART-1-specific). After 48 hours, transfected cells were cocultured with HLA-A2-positive cells and a titration of three separate Melan-A (MART-1) peptides: MART-1$^{Leu26-35}$ (**Panel A**), MART-1$^{26-35}$ (**Panel B**) and MART-1$^{27-35}$ (**Panel C**). MART-1$^{Leu26-35}$ contains a leucine at position 2 of the native sequence. After 6 hours, Bio-Glo-NL™ Reagent was added and luminescence quantified using the GloMax® Discover System. Data were fitted to four-parameter logistic curves using GraphPad Prism® software. The presence of the CD8 coreceptor enhanced the sensitivity of the DMF4 TCR to activate the TCRαβ-KO Cells.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
T Cell Activation Bioassay (TCRαβ-KO, CD8+), Propagation Model	**1 each**	**GA1162**

Not for Medical Diagnostic Use. Includes:

- 2 vials TCRαβ-KO (CD8+) (CPM), 1.0×10^7 cells/ml (1.0ml per vial)

PRODUCT	SIZE	CAT.#
T Cell Activation Bioassay (TCRαβ-KO, CD4+), Propagation Model	**1 each**	**GA1172**

Not for Medical Diagnostic Use. Includes:

- 2 vials TCRαβ-KO (CD4+) (CPM), 1.0×10^7 cells/ml (1.0ml per vial)

PRODUCT	SIZE	CAT.#
T Cell Activation Bioassay (TCRαβ-KO, CD4+, CD8+), Propagation Model	**1 each**	**GA1182**

Not for Medical Diagnostic Use. Includes:

- 2 vials TCRαβ-KO (CD4+ CD8+) (CPM), 1.0×10^7 cells/ml (1.0ml per vial)

PRODUCT	SIZE	CAT.#
T Cell Activation Bioassay (TCRαβ-KO, CD8+), Cell Bank	**1 each**	**GA1220**

Not for Medical Diagnostic Use. Includes:

- 50 vials TCRαβ-KO (CD8+), 1.0×10^7 cells/ml (1.0ml per vial)

PRODUCT	SIZE	CAT.#
T Cell Activation Bioassay (TCRαβ-KO, CD4+), Cell Bank	**1 each**	**GA1210**

Not for Medical Diagnostic Use. Includes:

- 50 vials TCRαβ-KO (CD4+), 1.0×10^7 cells/ml (1.0ml per vial)

PRODUCT	SIZE	CAT.#
T Cell Activation Bioassay (TCRαβ-KO, CD4+, CD8+), Cell Bank	**1 each**	**GA1230**

Not for Medical Diagnostic Use. Includes:

- 50 vials TCRαβ-KO (CD4+ CD8+) 1.0×10^7 cells/ml (1.0ml per vial)

Note: When using propagation model, 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 affect cell viability and cell performance.

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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.

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 for the specified product from the website such as Certificate of Analysis.

Note: The T Cell Activation Bioassay (TCRαβ-KO) uses Bio-Glo-NL™ Luciferase Assay System (Cat.# J3081, J3082, J3083) for detection. **Do not** use Bio-Glo™ Luciferase Assay System.

The T Cell Activation Bioassay (TCRαβ-KO), Propagation Model, is intended to be used with user-provided TCR constructs. TCRs can be introduced by transfection or transduction. An optimized method for plasmid-based transfection by electroporation is provided in this Technical Manual. However, we have had success using alternative methods of electroporation. We do not suggest lipid transfection with these cell lines.

We offer separately a TCR Transfection Positive Control Kit from Promega Tailored Solutions. (For information contact: **tailoredsolutions@promega.com**). This kit includes a Positive Control TCR Plasmid, a Positive Control Peptide and APCs in thaw-and-use format. We recommend including these reagents as a positive control in the first few assays to gain familiarity with the assay. Data generated using these reagents are shown in Section 9.A.

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 the TCR Transfection Positive Control Kit. You may need to adjust the parameters provided here and optimize assay conditions for your own TCR constructs and transfection or transduction method.

The T Cell Activation Bioassay (TCRαβ-KO) produces a bioluminescent signal and requires a sensitive luminescence plate reader. Bioassay development and performance data included in this Technical Manual were generated using the GloMax® Discover System (see Section 9.C, Related Products). An integration time of 0.5 second/well was used for all readings. The bioassay is compatible with most other plate-reading luminometers, though relative luminescence unit (RLU) readings will vary with the sensitivity and settings of each instrument. If using a reader with adjustable gain, we recommend a high-gain setting. The use of different instruments and gain adjustment will affect the magnitude of the raw data, but should not affect the relative potency of the TCR constructs tested.

3.A. Materials to Be Supplied by the User

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

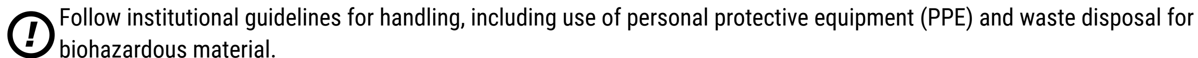
Reagents

- user-generated TCR expression plasmids or transduction particles
- user-defined antigenic proteins/peptides and APCs
- RPMI 1640 Medium with L-glutamine and HEPES (e.g., GIBCO® Cat.# 22400 105)
- fetal bovine serum (e.g., VWR Cat.# 89510-194 or HyClone Cat.# SH30071.03)
- hygromycin B (e.g., Invitrogen™ Cat.# 10687010)
- blasticidin S HCl (e.g., GIBCO® Cat.# A1113903) for the CD8+ and CD4+, CD8+ cell lines
- sodium pyruvate (e.g., GIBCO® Cat.# 11360070)
- MEM nonessential amino acids, 100X (e.g., GIBCO® Cat.# 11140050)
- DMSO (e.g., Sigma Cat.# D2650)
- Trypan blue solution (e.g., Sigma Cat.# T8154)
- Bio-Glo-NL™ Luciferase Assay System (Cat.# J3081, J3082, J3083)
- DPBS (e.g., GIBCO® Cat.# 14190250)
- **optional:** TCR Transfection Positive Control Kit (e-mail: **tailoredsolutions@promega.com**)
- **optional:** Ingenio Electroporation Solution (Mirus Cat.# MIR 50111)

Supplies and Equipment

- 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 dilution reservoirs (e.g., Dilux® Cat.# D-1003) for preparing antigenic peptide 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 humidified incubator
- 37°C water bath
- sensitive plate reader with glow luminescence measuring capability or luminometer (e.g., GloMax® Discover System, Cat.# GM3000, or equivalent system)
- **optional:** Bio-Rad GenePulser Xcell (Bio-Rad Cat.# 1652661)

4. Preparing TCRαβ-KO Cells



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

Note: The cell thawing, propagation and banking instructions are applicable for TCRαβ-KO (CD4+) Cells, TCRαβ-KO (CD8+) Cells and TCRαβ-KO (CD4+, CD8+) Cells. See Section 9.B, Composition of Buffers and Solutions, for preparation of needed media.

4.A. Cell Thawing and Initial Cell Culture

1. Prepare 25ml of initial cell culture medium by adding 2.5ml of FBS to 22.5ml 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 TCRαβ-KO 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 × *g* for 10 minutes.
6. Carefully aspirate the medium and resuspend the cell pellet in 15ml 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% 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 after thawing, at which time cell viability is typically >90%, and the average cell doubling rate is 22–30 hours. Passage number should be recorded for each passage. In our experience, cells maintain their functionality for up to 25 passages, or 54 cell doublings, if passaging is performed on a Monday-Wednesday-Friday schedule.

Cell Passage Schedule	Cell Seeding Density
2 days	4.0×10^5 cells/ml
3 days	2.5×10^5 cells/ml

1. On the day of cell passage, measure cell viability and density by Trypan blue staining.
2. Seed the cells at a density of 4×10^5 cells/ml if passaging every two days (e.g., Monday-Wednesday or Wednesday-Friday) or 2.5×10^5 cells/ml if passaging every three days (e.g., Friday-Monday). Always maintain the flasks in a horizontal position in the incubator. Do not allow the cells to grow to a density greater than 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 in a humidified 37°C, 5% CO_2 incubator.

4.C. Cell Freezing and Banking

1. On the day of cell freezing, prepare fresh cell freezing medium and keep on ice.
2. Gently mix the cells with a pipette to create a homogenous cell suspension.
3. Remove a sample for cell counting by Trypan blue staining. Calculate the volume of cell freezing medium needed based on desired cell freezing densities of 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 × *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. Assay Protocol for Plasmid-Based TCR Transfection

This protocol describes transfection reactions of a plasmid encoding a TCR alpha and beta chain of interest, as well as a mock (no plasmid) transfection control. This protocol can be scaled to include multiple transfections of the same plasmid or of different plasmids in separate transfection reactions.

The plasmid from the TCR Transfection Positive Control Kit was used as the example for this protocol. Other transfection or transduction reagents and protocols are possible but will require optimization.

5.A. Preparing Cell Recovery Medium, Electroporation Buffer and TCR Plasmid

1. **Cell Recovery Medium:** On the day of transfection, prepare 20ml of cell recovery medium (90% RPMI 1640/10% FBS). Transfer 8ml to each of two T25 flasks, and place the flasks in a 37°C, 5% CO_2 incubator. Warm remaining cell recovery medium to 37°C before use.
2. **Electroporation Buffer:** On the day of transfection, equilibrate the Ingenio Electroporation Solution to room temperature on the bench top.
3. **TCR Plasmid:** On the day of transfection, remove TCR plasmid from storage at –30°C to –10°C and thaw on ice.

5.B. Transfecting TCRαβ-KO Cells with the Bio-Rad Gene Pulser Xcell Device

While maintaining the TCRαβ-KO Cells, it is important to follow the recommended cell seeding density. Changes in cell culture volume or seeding density can 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 90%.

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

1. Passage the cells two days before performing the transfection as described in Section 4.B.
2. Count the TCRαβ-KO Cells by Trypan blue staining and calculate the cell density and viability.
3. Transfer 1.2×10^7 TCRαβ-KO Cells to a 15ml conical tube.
4. Pellet the cells at 130 × *g* for 10 minutes at ambient temperature, aspirate medium and resuspend the pellet in 10ml of DPBS to wash.
5. Pellet the cells at 130 × *g* for 10 minutes at ambient temperature, aspirate DPBS and resuspend the pellet in 500µl of Ingenio Electroporation Solution.
6. Add 250µl of cells to each of two sterile microcentrifuge tubes.
7. Add 10µl (20µg) of Positive Control TCR Plasmid to one of the tubes. Mix well by pipetting. Mock transfected control cells will not get plasmid.
8. Transfer the cells to fresh 4mm cuvettes.
9. Electroporate the cuvettes in a Bio-Rad Gene Pulser Xcell Device using the exponential decay settings at 260V, 950µF and ∞Ω.
10. Add 0.5ml of warm cell recovery medium to each cuvette. Using a sterile transfer pipette, gently transfer the entire contents of the cuvettes to the T25 flasks containing 8ml of cell recovery medium.
11. Place the T25 flasks in a humidified 37°C, 5% CO_2 incubator for 48 hours.

6. T Cell Activation Bioassay

This assay protocol describes testing of mock-transfected and TCR plasmid-transfected TCRαβ-KO Cells by activation with cognate peptide and APCs. The MHCII APC Cells and Positive Control Peptide from the TCR Transfection Positive Control Kit were used as the APCs and antigenic peptide for this protocol. Other APCs and protein/peptide antigens can be used but will require optimization.

6.A. Preparing Assay Buffer, Antigenic Peptides and Bio-Glo-NL™ Reagent

1. **Assay Buffer**: On the day of the assay, prepare an appropriate amount of assay buffer (99% RPMI 1640/1% FBS). Mix well and warm to 37°C prior to use.
2. **Antigenic Peptides:** Using assay buffer as the diluent, prepare starting dilutions (dilu1, 2X final concentration) of antigenic peptides.

   Note: For the Positive Control Peptide, thaw the peptide at room temperature. Prepare 1 ml of 2μg/ml starting dilution (dilu1, 2X final concentration) by adding 1μl of Positive Control Peptide stock (2mg/ml) to 999μl of assay buffer.
3. **Bio-Glo-NL™ Reagent:** For reference, 10ml of Bio-Glo-NL™ Reagent is sufficient to assay 120 wells in a 96-well assay format. Store the Bio-Glo-NL™ Luciferase Assay Substrate at –20°C. Thaw the Bio-Glo-NL™ Luciferase Assay Buffer at room temperature (do not exceed 25°C) during the 6-hour assay induction. We recommend preparing the reconstituted Bio-Glo-NL™ Reagent immediately before use.

Note: The T Cell Activation Bioassay (TCRαβ-KO) is compatible only with Bio-Glo-NL™ Luciferase Assay System (Cat.# J3081, J3082, J3083). **Do not** use the Bio-Glo™ Luciferase Assay System.

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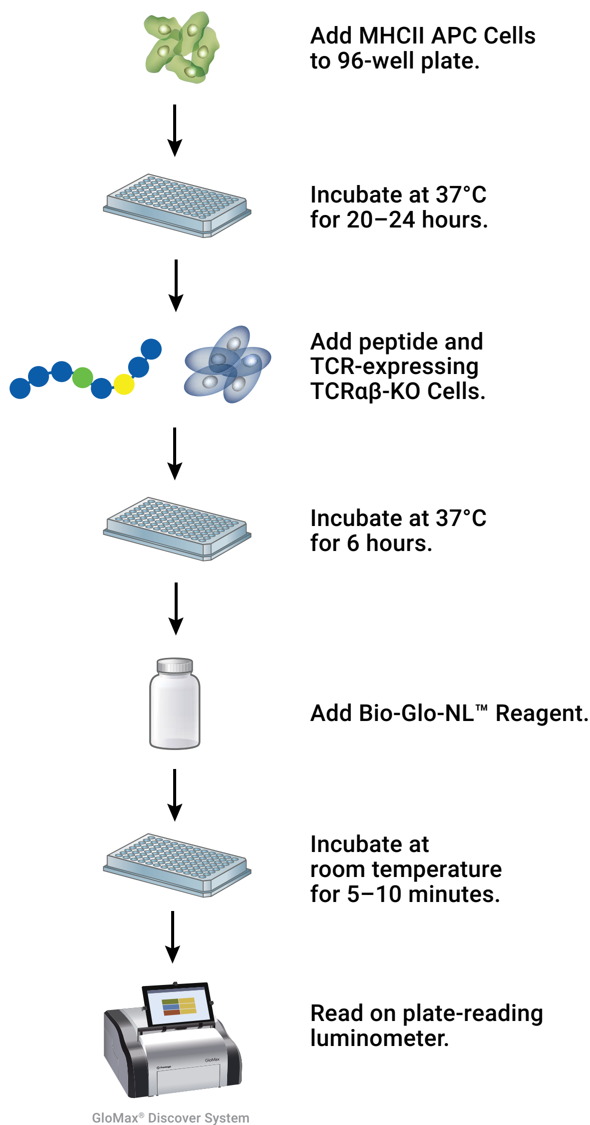

Figure 7. Schematic protocol for the T Cell Activation Bioassay (TCRαβ-KO).

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6.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 (n = 3) dilutions of Positive Control Peptide to generate ten-point dose-response curves for mock-transfected and Positive Control TCR-transfected T Cell Activation Bioassay (TCRαβ-KO) cells.

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 peptide	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Mock Transfected
C	B	no peptide	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	TCR Transfected
D	B	no peptide	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Mock Transfected
E	B	no peptide	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	TCR Transfected
F	B	no peptide	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	Mock Transfected
G	B	no peptide	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1	B	TCR Transfected
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 mock-transfected and Positive Control TCR-transfected cells and wells containing assay buffer (denoted by "B") alone.

6.C. Plate MHCII APC Cells

The MHCII APC Cells contained in the TCR Transfection Positive Control Kit are provided in thaw-and-use format, which includes a vial of cells for single use, along with DMEM and FBS. There are sufficient cells in the vial for two 96-well assay plates in inner 60-well format. Other APCs, both adherent and suspension, can be used but will require optimization of cell number and incubation times.

1. On the day before performing the assay, add 1.5ml of FBS to 13ml of DMEM in a 50ml conical tube to make 14.5ml of APC plating medium (90% DMEM/10% FBS). Warm in a 37°C water bath.
2. Remove a vial of MHCII APC 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 minutes). While thawing, gently agitate and visually inspect.

6.C. Plate MHCII APC Cells (continued)

3. Gently mix the cell suspension by pipetting, then transfer the cells (0.5ml) to the 50ml conical tube containing 14.5ml of prewarmed APC plating medium. Mix well by gently inverting.
4. Transfer the cell 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, tissue culture-treated assay plate.
5. Add 100µl of prewarmed (37°C) DMEM to each of the outside wells of the assay plate.
6. Cover the assay plate with a lid and incubate the cells overnight (20–24 hours) in a humidified 37°C, 5% CO_2 incubator.

6.D. Preparing Peptide Serial Dilutions

The instructions described here are for preparing a 3.5-fold serial dilution of Positive Control Peptide for analysis in triplicate. For other dilution schemes, adjust the volumes accordingly.

1. On the day of the assay, prepare an appropriate amount of assay buffer as described in Section 6.A.
2. To a sterile dilution reservoir, add 700µl of Positive Control Peptide starting dilution (dilu1, 2X final concentration) to well 11 (see Figure 9).
3. Add 500µl of assay buffer to the other wells, from well 10 to well 2.
4. Transfer 200µl of the peptide starting dilution from well 11 to well 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent 3.5-fold serial dilutions across the dilution reservoir from right to left through well 3. Do not dilute into well 2.
6. Cover the reservoir with a lid and keep at ambient temperature (22–25°C) while preparing the transfected T Cell Activation Bioassay (TCRαβ-KO) Cells.

Recommended Dilution Reservoir Layout for Positive Control Peptide.

	1	2	3	4	5	6	7	8	9	10	11	12	
A		no peptide	dilu9	dilu8	dilu7	dilu6	dilu5	dilu4	dilu3	dilu2	dilu1		Positive Control Peptide

Figure 9. Example layout showing serial dilution of the Positive Control Peptide.

6.E. Preparing and Plating TCRαβ-KO Cells

1. Count the transfected TCRαβ-KO Cells by Trypan blue staining and calculate the cell density and viability.
2. Transfer cells to conical centrifuge tubes and pellet the cells at 130 × *g* for 10 minutes at ambient temperature.
3. Remove medium and resuspend the cells in assay buffer to achieve a final cell density of 1×10^6 cells/ml.
4. Remove the 96-well assay plate containing MHCII APC Cells from the incubator. Invert the assay plate above a sink to remove the medium. Then, place the inverted plate on a paper towel for 5–10 seconds to drain any remaining medium from each of the wells. Do not use a vacuum aspirator to remove the medium.
5. Transfer the mock- and TCR-transfected TCRαβ-KO Cells to sterile reagent reservoirs. Using a multichannel pipette, add 40µl of the appropriate cells to the preplated MHCII APC Cells according to the plate layout in Figure 8.
6. Using a multichannel pipette, add 40µl of the Positive Control Peptide dilutions (prepared in Section 6.D) to the wells containing MHCII APC Cells and TCRαβ-KO Cells.
7. Add 80µl of assay buffer to each of the outside wells of the assay plate.
8. Cover the assay plate with a lid and place in a 37°C, 5% CO_2 incubator for 6 hours.

6.F. Preparing and Adding Bio-Glo-NL™ Reagent

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

Note: The T Cell Activation Bioassay (TCRαβ-KO) is compatible only with the Bio-Glo-NL™ Luciferase Assay System (Cat.# J3081, J3082). **Do not** use the Bio-Glo™ Luciferase Assay System.

1. Remove the Bio-Glo-NL™ Luciferase Assay Substrate from –20°C storage and mix by pipetting. Briefly centrifuge the tubes if the substrate has collected in the cap or on the sides of the tubes.
2. Prepare the desired amount of reconstituted Bio-Glo-NL™ Reagent by combining one volume of substrate with 50 volumes of buffer. For example, if the experiment requires 10ml of reagent, add 200µl of substrate to 10ml of buffer. Ten milliliters (10ml) of Bio-Glo-NL™ Reagent is sufficient for 120 wells (two assay plates, using the inner 60 wells of each plate).
3. Remove assay plates from the incubator after the incubation period and equilibrate to room temperature for 10–15 minutes.
4. Using a manual multichannel pipette, add 80µl of Bio-Glo-NL™ Reagent to the inner 60 wells of the assay plates, taking care not to create bubbles.
5. Add 80µl of Bio-Glo-NL™ Reagent to wells B1, D1 and F1 of each assay plate to measure background signal.
6. Wait 5–10 minutes, then measure the luminescence in a GloMax® Discover System or a plate reader with glow-type luminescence reading capabilities. The luminescence intensity will decay gradually, with a signal half-life of approximately 120 minutes at room temperature.

   Note: Varying the Bio-Glo-NL™ Reagent incubation time will affect the raw relative light unit (RLU) values but should not significantly change the EC_{50} value and maximum fold induction.

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6.G. Data Analysis

1. Determine the plate background by calculating the average RLU from wells B1, D1 and F1.

2. Calculate fold induction = $$\frac{\text{RLU (induced–background)}}{\text{RLU (no peptide control–background)}}$$

3. Graph data as RLU versus Log_{10} [peptide] and fold induction versus Log_{10} [peptide]. Fit curves and determine the EC_{50} value of 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 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. Optimize transfection/transduction methods to ensure an adequate number of viable cells for the assay.
	Low activity of Bio-Glo-NL™ Reagent leads to low RLU. Store and handle the Bio-Glo-NL™ Reagent according to the instructions.
	Ensure that you are using Bio-Glo-NL™ Reagent in the assay. The T Cell Activation Bioassay (TCRαβ-KO) is not compatible with Bio-Glo™ Reagent.
Weak assay response (low fold induction)	Optimize the concentration range of your antigenic protein(s)/peptide(s) to achieve a dose response with complete upper and lower asymptotes. The EC_{50} value obtained in the T Cell Activation Bioassay (TCRαβ-KO) may vary from the EC_{50} value obtained using other methods such as primary T cell-based assays.
	Ensure surface expression of transfected TCR constructs by flow cytometry. Differences in surface TCR expression can be observed based on TCR sequence and plasmid construction, as well as transfection/transduction method. Optimize transfection/transduction methods and TCR plasmid design to ensure adequate TCR surface expression.

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7. Troubleshooting (continued)

Symptoms	Possible Causes and Comments
Variability in assay performance	Variations in cell growth conditions including cell plating, harvest density, cell viability and cell doubling time can cause variable transfection/transduction efficiency and assay performance. Avoid one-day cell passages whenever possible. Do not transfect cells after a one-day passage. 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 poor 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.
	Reliable methods of TCR gene transfer (transfection or transduction) are required for consistent assay performance.
	Use of the CD4+ or CD8+ TCRαβ-KO Cells can result in variable EC_{50} response depending on the TCR construct being tested.
	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.

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8. References

1. United States Food and Drug Administration "Approved Cellular and Gene Therapy Products" web page: **www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products** (Internet; accessed April 2, 2021).
2. van Loenen, M.M. *et al.* (2010) Mixed T cell receptor dimers harbor potentially harmful neoreactivity. *Proc. Natl. Acad. Sci. USA* **107**, 10972–7.
3. Ahmadi, M. *et al.* (2011) CD3 limits the efficacy of TCR gene therapy in vivo. *Blood* **118**, 3528–37.
4. Legut, M. *et al.* (2018) CRISPR-mediated TCR replacement generates superior anticancer transgenic T cells. *Blood* **131**, 311–22.
5. Hebeisen, M. *et al.* (2013) Molecular insights for optimizing T cell receptor specificity against cancer. *Front. Immunol.* **4**, 154.
6. Zhong, S. *et al.* (2013) T-cell receptor affinity and avidity defines antitumor responses and autoimmunity in T-cell immunotherapy. *Proc. Natl. Acad. Sci. USA* **110**, 6973–8.
7. Labrecque, N. *et al.* (2001) How much TCR does a T cell need? *Immunity* **15**, 71–82.
8. Thomas, S. *et al.* (2010) Molecular immunology lessons from therapeutic T-cell receptor gene transfer. *Immunology* **129**, 170–7.

9. Appendix

9.A. Representative Assay Results

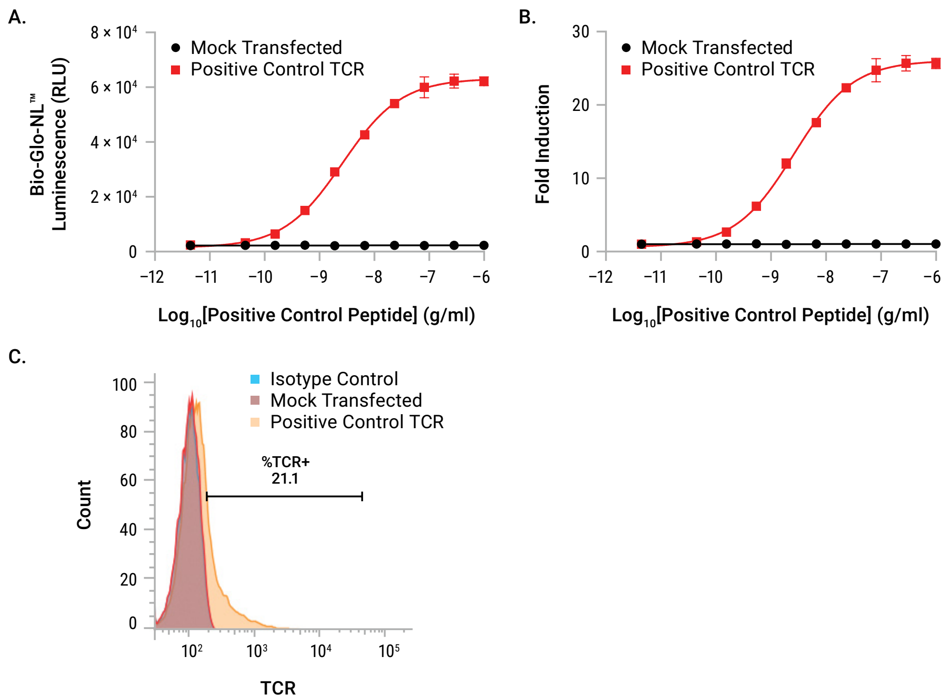

Figure 10. Representative data of the T Cell Activation Bioassay (TCRαβ-KO, CD8+, Cat.# GA1162). Panels A and B. TCRαβ-KO (CD8+) Cells were transfected with the Positive Control TCR Plasmid (or mock transfected) using the Lonza Nucleofector™ 2b Device. Forty-eight hours after transfection, the cells were cocultured with MHCII APC Cells and a titration of the Positive Control Peptide. After a 6-hour induction at 37°C, Bio-Glo-NL™ Reagent was added and luminescence measured using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. The EC_{50} was 2.6ng/ml and the fold induction was 26 for the Positive Control TCR-transfected cells. **Panel C.** Flow cytometric analysis of surface TCR expression. TCRαβ-KO (CD8+) Cells were transiently transfected as described in Panel A. Forty-eight hours after transfection, cells were labeled with fluorochrome-conjugated anti-TCR antibodies (clone IP26) and analyzed on a BD LSRFortessa™ X-20 flow cytometer. Data analysis was performed with FlowJo™ software.

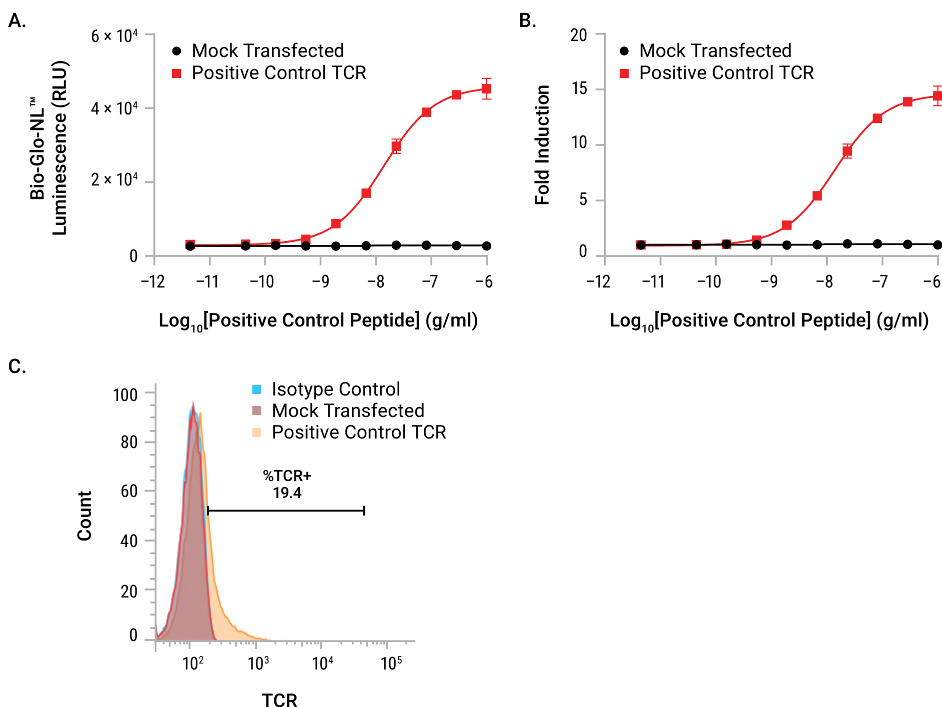

Figure 11. Representative Data of the T Cell Activation Bioassay (TCRαβ-KO, CD4+, Cat.# GA1172). Panels A and B. TCRαβ-KO (CD4+) Cells were transfected with the Positive Control TCR Plasmid (or mock transfected) using the Lonza Nucleofector™ 2b Device. Forty-eight hours after transfection, the cells were cocultured with MHCII APC Cells and a titration of the Positive Control Peptide. After a 6-hour induction at 37°C, Bio-Glo-NL™ Reagent was added and luminescence measured using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. The EC_{50} was 14ng/ml and the fold induction was 15 for the Positive Control TCR-transfected cells. **Panel C.** Flow cytometric analysis of surface TCR expression. TCRαβ-KO (CD4+) Cells were transiently transfected as described in Panels A and B. Forty-eight hours after transfection, cells were labeled with fluorochrome-conjugated anti-TCR antibodies (clone IP26) and analyzed on a BD LSRFortessa™ X-20 flow cytometer. Data analysis was performed with FlowJo™ software.

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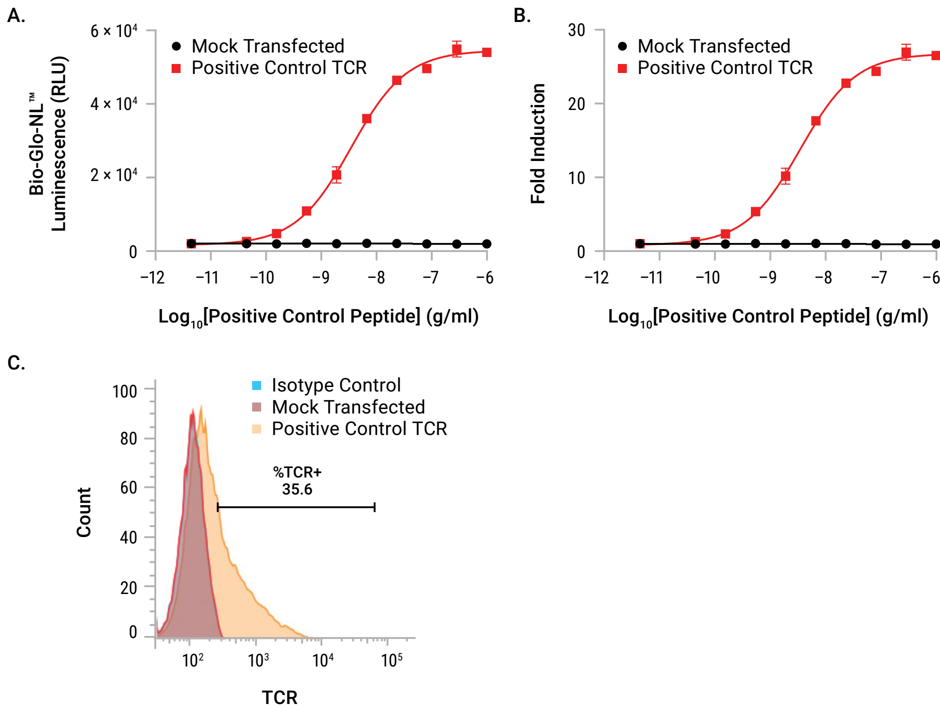

Figure 12. Representative Data of the T Cell Activation Bioassay (TCRαβ-KO, CD4+ CD8+, Cat.# GA1182). Panels A and B. TCRαβ-KO (CD4+, CD8+) Cells were transfected with the Positive Control TCR Plasmid (or mock transfected) using the Lonza Nucleofector™ 2b Device. Forty-eight hours after transfection, the cells were cocultured with MHCII APC Cells and a titration of the Positive Control Peptide. After a 6-hour induction at 37°C, Bio-Glo-NL™ Reagent was added and luminescence measured using the GloMax® Discover System. Data were fitted to a four-parameter logistic curve using GraphPad Prism® software. The EC_{50} was 3.5ng/ml and the fold induction was 27 for the Positive Control TCR-transfected cells. **Panel C.** Flow cytometric analysis of surface TCR expression. TCRαβ-KO (CD4+, CD8+) Cells were transiently transfected as described in Panels A and B. Forty-eight hours after transfection, cells were labeled with fluorochrome-conjugated anti-TCR antibodies (clone IP26) and analyzed on a BD LSRFortessa™ X-20 flow cytometer. Data analysis was performed with FlowJo™ software.

9.B. Composition of Buffers and Solutions

initial cell culture medium for TCRαβ-KO Cells

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

cell growth medium for TCRαβ-KO (CD4+) Cells

90%	RPMI 1640 with L-glutamine and HEPES
10%	FBS
400μg/ml	hygromycin B
1mM	sodium pyruvate
0.1mM	1X MEM nonessential amino acids

cell freezing medium for TCRαβ-KO Cells

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

cell growth medium for TCRαβ-KO (CD8+) Cells and TCRαβ-KO (CD4+, CD8+) Cells

90%	RPMI 1640 with L-glutamine and HEPES
10%	FBS
400μg/ml	hygromycin B
1mM	sodium pyruvate
0.1mM	1X MEM nonessential amino acids
10μg/ml	blasticidin S HCl

assay buffer

99%	RPMI 1640 with L-glutamine and HEPES
1%	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

*For Research Use Only. Not for use in diagnostic procedures.
**Not for Medical Diagnostic Use.
Additional kit formats are available.

9.C. Related Products (continued)

Fc Effector Immunoassay

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

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

Immune Checkpoint Bioassays

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

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T Cell Activation Bioassays

Product	Size	Cat.#
T Cell Activation Bioassay (IL-2)	1 each	J1651
T Cell Activation Bioassay (NFAT)	1 each	J1621

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Cytokine and Growth Factor Bioassays

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

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

Control Antibodies and Proteins

Product	Size	Cat.#
Control Ab, Anti-4-1BB	50µg	K1161
Control Ab, Anti-CD20	5 µg	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	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.

9.C. Related Products (continued)

Luminometers

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

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

10. Summary of Changes

The following change was made to the 12/22 revision of this document:

1. Updated Sections 3 and 9.C.
2. Revised Section 5, including Section 5.B title.
3. Replaced document font.

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3) Any in vivo veterinary or livestock use;

4) The development, manufacture (including any bioproduction), distribution, importation, exportation, transportation, sale, offer for sale, marketing, promotion or other exploitation or use of the Intellectual Property or the material or derivatives as a therapeutic or diagnostic for humans or animals;

5) Products that provide nutritional benefits and are regulated by a regulatory authority as a drug or biologic pursuant to Section 505 of the Federal Food, Drug, and Cosmetic Act of 1938, as amended, Section 351 of the Public Health Service Act of 1944, as amended, or any successor laws, or equivalent laws or regulations in jurisdiction outside the United States;

6) Any agricultural use, including but not limited to, the use or application in the cultivation, growth, manufacture, exportation, or production of any tobacco product; and

7) Any use or application relating to gene drive.

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Appendix A: Broad IP List

BROAD TECHID	APPLICANTS	EXEMPLARY FAMILY SERIAL NO
BI-2011/008	Broad, MIT, Harvard and Rockefeller	PCT/US2013/074611; WO2014093595
BI-2011/008B	Broad, MIT and Harvard	14/259,420
BI-2011/008A	Broad and MIT	PCT/US2013/074743; WO2014093661
BI-2011/008C	Broad, MIT, and Harvard	PCT/US2013/074790; WO2014093694
BI-2011/020	Broad, MIT and Harvard	PCT/US2013/051418; WO2014018423
BI-2012/084A	Broad and MIT	PCT/US2013/074825; WO2014093718
BI-2012/084B	Broad, MIT and Harvard	PCT/US2013/074812; WO2014093709
BI-2013/003	Broad, MIT and Harvard	PCT/US2013/074667; WO2014093622
BI-2013/003D	Broad, MIT and Harvard	PCT/US2014/041803; WO2014201425
BI-2013/004E	Broad, MIT and Harvard	PCT/US2013/074691; WO2014093635
BI-2013/004F	Broad, MIT and Harvard	PCT/US2013/074736; Wo2014093655
BI-2013/004G	Broad, MIT and Harvard	PCT/US2013/074819; WO2014093712
BI-2013/007	Broad, MIT and Harvard	14/855,046; US20160068822
BI-2013/066	Broad, MIT and Harvard	PCT/US2014/041800; WO2014204724
BI-2013/073	Broad and MIT	PCT/US2014/041806; WO2014204727
BI-2013/085	Broad, MIT and Whitehead	15/141,348; US20160251648
BI-2013/087J	Broad, Editas*, Iowa and MIT	PCT/US2014/064663; WO2015070083
BI-2013/087M	Broad, Iowa and MIT	PCT/US2014/069902;WO2015089354
BI-2013/087V	Broad, Iowa and MIT	PCT/US2014/069897; WO2015089351
BI-2013/093	Broad, MIT and Tokyo	15/171,141; US20160340660
BI-2013/094	Broad, MIT and Rockefeller	PCT/US2014/070135; Wo2015089465
BI-2013/098	Broad and MIT	PCT/US2014/070068; WO2015089427
BI-2013/099	Broad, MIT and Harvard	PCT/US2014/041804; WO2014204726
BI-2013/101	Broad and MIT	PCT/US2014/070127; WO2015089462
BI-2013/103	Broad and MIT	PCT/US2014/041808; WO201404728

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BROAD TECHID	APPLICANTS	EXEMPLARY FAMILY SERIAL NO
BI-2013/105	Broad, MIT and Harvard	PCT/US2014/041809; WO2014204729
BI-2013/107	Broad and MIT	PCT/US2014/070057; WO2015089419
BI-2013/112	Broad and MIT	PCT/US2013/074800; WO2014093701
BI-2013/113	Broad, MIT and Harvard	PCT/US2014/070152; WO2015089473
BI-2014/005	Broad, MIT, Harvard and Tokyo	PCT/US2014/070175; WO2015089486
BI-2014/061	Broad, MIT and Harvard	PCT/US2015/045504; WO2016028682
BI-2014/069	Broad and MIT	15/467,888; US20180010134
BI-2014/071	Broad and MIT	15/349,603; US20170107536
BI-2014/072	Broad and MIT	15/467,949; US20180044662
BI-2014/084	Broad, MIT and Harvard	15/469,081; US20180057810
BI-2014/097	Broad, MIT and Harvard	PCT/US2015/067177; WO2016106244
BI-2014/100	Broad, MIT and Harvard	PCT/US2015/065385; WO2016094867
BI-2014/101	Broad, MIT and Harvard	15/632,067; US20170306335
BI-2014/103	Broad and MIT	PCT/US2015/067138; WO2016100974
BI-2014/106	Broad and MIT	PCT/US2015/065393; WO2016094872
BI-2014/107	Broad and MIT	15/619,735; US20170349894
BI-2014/108	Broad and MIT	15/619,737; US20170349914
BI-2014/113	Broad and MIT	15/640,103; US20180112255
BI-2015/002	Broad, MIT, Harvard and Tokyo	PCT/US2016/038252; WO2016205759
BI-2015/052	Broad and MIT	PCT/US2016/038034; WO2016205613
BI-2015/053	MIT	PCT/US2016/038205; WO2016205728
10086	Broad and MIT	PCT/US2017/047458; WO2018035387
10114	Broad and MIT	PCT/US2017/053795; WO2018064208
10125	Broad and MIT	62/502,064 62/564,102
10209	Broad, Harvard, MIT, New York University and NY Genome Center	62/529,573

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[g]Product cannot be used for proficiency testing.

[h]Patent Pending.

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