



TECHNICAL MANUAL

IL-15 Bioassay

Instructions for Use of Products
JA2011 and JA2015

IL-15 Bioassay

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

Interleukin-15 (IL-15), discovered in 1994 as a T cell growth factor, is a small 14.5kDa monomer secreted by a variety of cell types including monocytes, macrophages, dendritic and epithelial cells (1,2). IL-15 promotes activation and expansion of natural killer (NK), natural killer T (NKT) and CD8+ memory T cells. Unlike IL-2, IL-15 does not affect CD4+ regulatory T cells (Treg) or induce activation-induced cell death (AICD).

The IL-15 receptor (IL-15R) is a type 1 cytokine receptor, consisting of 3 subunits: IL-15R α (CD215), which contains a structural binding sushi domain at the N terminus, IL-2/15R β (CD122) and IL-15R γ (CD132). IL-15R γ is also known as the γ common chain (γ c) and is found in receptors for IL-4, IL-7, IL-9, IL-15, and IL-21. The IL-15 and IL-2 receptors share β and γ c subunits, with cytokine specificity resulting from the respective α subunit. In contrast to IL-2, which has a relatively low affinity for its α receptor subunit, IL-15 has extremely high affinity for the IL-15R α .

Current understanding of IL-15 suggests the cytokine can bind and signal effector cells in three different ways. It predominantly signals in membrane form for a wide variety of cells that express both IL-15 and IL-15R α , including monocyte, macrophage and dendritic cells (3). In this scenario, IL-15R α chaperones IL-15 from the ER to the surface where it can trans present to a variety of IL-15R β γ expressing cells (NK, CD8+ T, NKT and B cells; 4). A soluble form of the heterodimer receptor/cytokine complex can also be secreted for cytokine presentation to IL-15R β γ -expressing effector cells (5). Finally, although monomeric IL-15 is rarely detected, it can bind directly to the IL-15R β γ , but with lower affinity.

In some situations, a soluble form of the entire extracellular IL-15R α receptor subunit can be released, bind IL-15 and function as an antagonist to trimeric receptor binding, because it sterically hinders IL-15 presentation. However, the extracellular sushi domain of IL-15R α can be engineered into an IL-15 fusion protein, giving the cytokine significantly improved stability and enhanced potency (6).

To counter a very short serum half-life (<1 hour), IL-15 therapeutic development has focused on extending the duration of signaling. This includes a number of fusion complexes composed of the aforementioned IL-15 and IL-15R α sushi (RLI-15, ALT-803, XmAb24306 and Deep IL-15) which mimic cytokine trans presentation. In addition, other therapeutic modalities have employed stabilized cytokine multimers (NKTR-255).

Multiple pathways can be activated by IL-15 signaling. In lymphocytes, JAK/STAT signaling begins with JAK1 and JAK3 tyrosine kinase recruitment and activation at the receptor cytoplasmic domains. These kinases recruit and activate STAT3 and 5 with phosphorylated dimer/tetramer translocation to the nucleus for transcriptional activation of a variety of proteins including Bcl-2, c-myc, c-fos and c-jun. In a second pathway, Shc adapter protein recruitment to the IL-2/15R β subunit occurs with activation of Grb2. Grb2 is in the PI3K pathway and can ultimately phosphorylate Akt, or it can activate RAS-RAF and finally MAPK. These pathways impact cell proliferation, anti-apoptotic survival and cytotoxic effector functions.

The IL-15 Bioassay^(a-e) (Cat. # JA2011, JA2015) is a bioluminescent cell-based assay designed to measure IL-15 stimulation or inhibition. The IL-15 Bioassay Cells are provided in a thaw-and-use format as cryopreserved cells that can be thawed, plated and used in an assay without the need for cell propagation. The IL-15 Bioassay Cells are also available in a Cell Propagation Model (CPM) format, which includes cryopreserved cells that can be thawed, propagated and banked for long-term use (IL-15 Bioassay, Propagation Model Cat. # J2962).

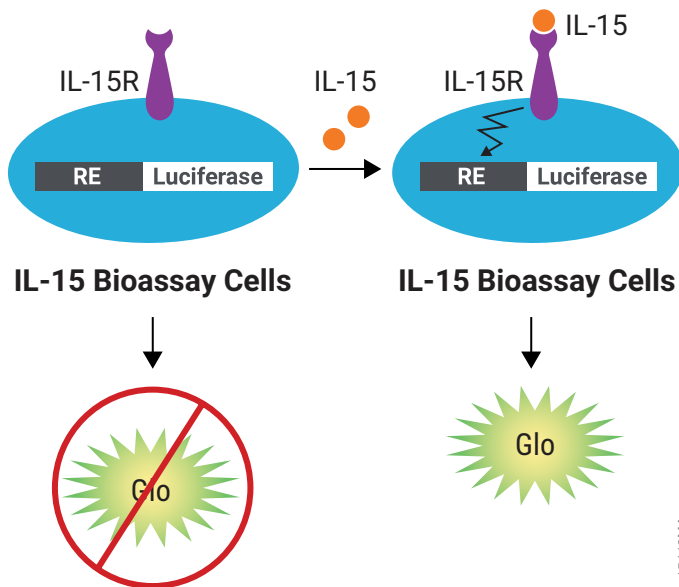


Figure 1. Representation of the IL-15 Bioassay. The IL-15 Bioassay consists of a genetically engineered cell line, IL-15 Bioassay Cells. When IL-15 binds to its receptor, receptor-mediated pathway signaling induces luminescence that can be detected upon addition of Bio-Glo™ Reagent and quantified with a luminometer. In the absence of IL-15, no signaling occurs downstream of IL-15R and a luminescent signal is not generated.

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1. Description (continued)

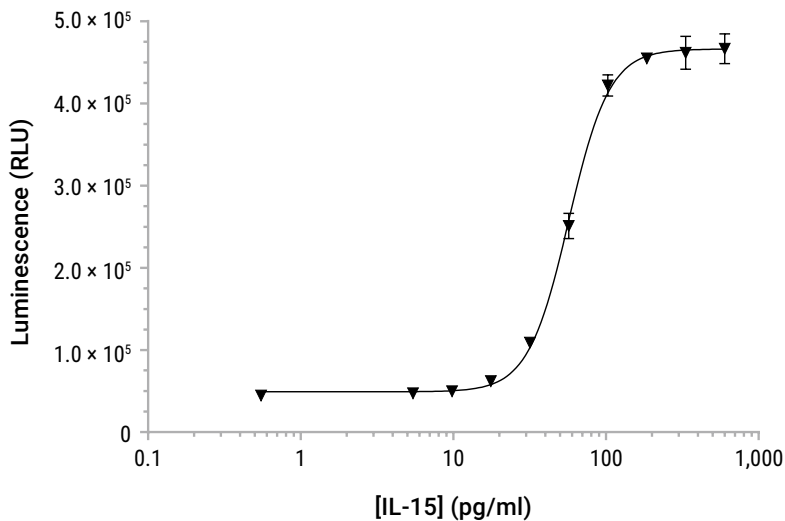


Figure 2. The IL-15 Bioassay is responsive to recombinant IL-15. IL-15 Bioassay Cells were prepared as described in this protocol and incubated with serial dilutions of recombinant IL-15. After a 6-hour incubation, Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. The EC₅₀ was 57pg/ml, with a fold induction of 10.48. Data were generated using thaw-and-use cells.

Table 1. The IL-15 Bioassay Shows Precision, Accuracy and Linearity.

| Parameter | Results | |
|--|-----------------------------|------------|
| Accuracy | % Expected Relative Potency | % Recovery |
| | 50 | 97.8 |
| | 70 | 101.4 |
| | 140 | 97.9 |
| | 200 | 102.7 |
| Repeatability (% CV) | 100% (Reference) | |
| Intermediate Precision (% CV) | 6.75 | |
| Linearity (r^2) | 0.998 | |
| Linearity ($y = mx + b$) | $y = 1.03x - 2.79$ | |
| <p>A 50–200% theoretical potency series of recombinant human IL-15 was analyzed in triplicate in three independent experiments performed on three days by two analysts (for a total of six independent experiments). 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.</p> | | |

1. Description (continued)

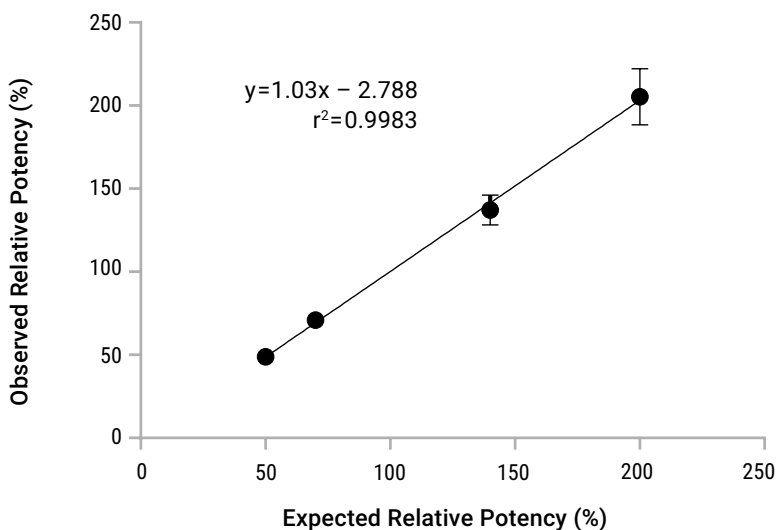


Figure 3. The IL-15 Bioassay shows precision, accuracy, and linearity. A 50–200% theoretical potency series of recombinant human IL-15 was analyzed in triplicate in three independent experiments performed on three days by two analysts using the IL-15 Bioassay (for a total of six independent experiments). Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Linearity and r^2 values were determined using GraphPad Prism® software. Data were generated using thaw-and-use cells.

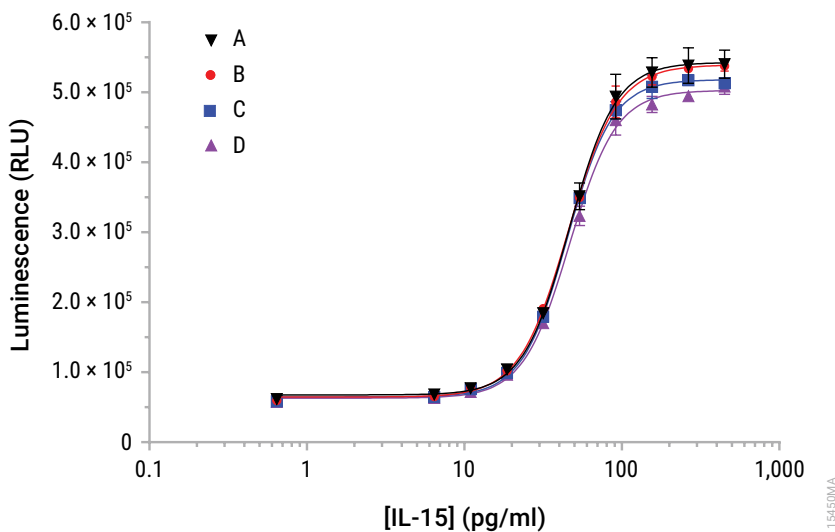


Figure 4. The IL-15 Bioassay demonstrates repeatability. Four separate serial dilution series of recombinant human IL-15 were analyzed on four individual assay plates using the IL-15 Bioassay. Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. The EC₅₀ values were 46.43, 45.79, 45.10 and 46.63pg/ml on the four assay plates. Data were generated using thaw-and-use cells.

1. Description (continued)

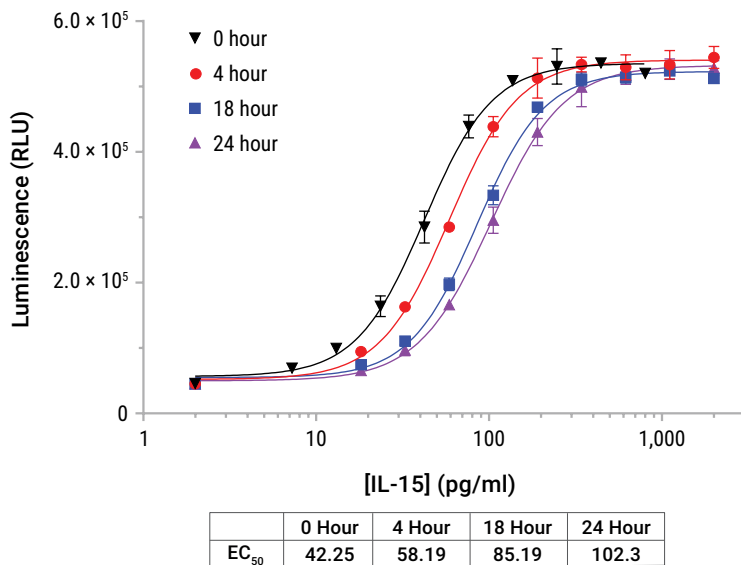
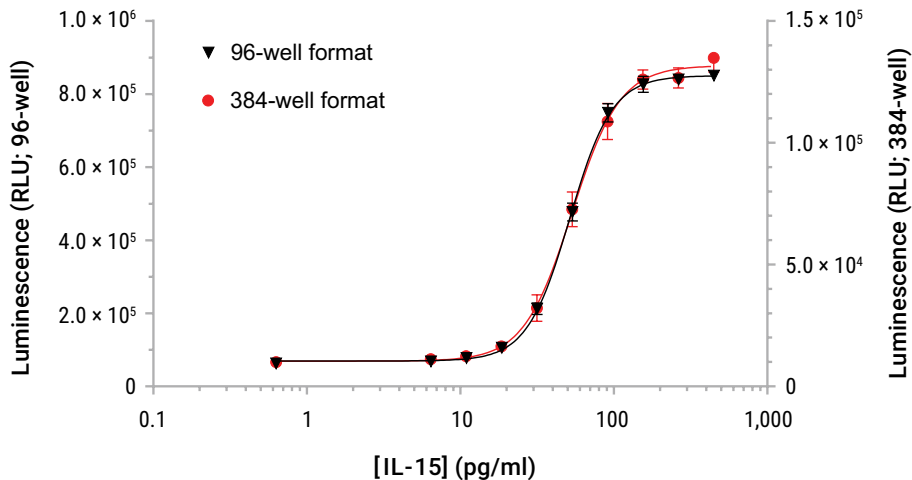


Figure 5. The IL-15 Bioassay indicates stability. Recombinant human IL-15 (PeproTech, 10µg/ml with BSA carrier) was heat stressed at 53°C for 0–24 hours prior to being tested in the IL-15 Bioassay. Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.

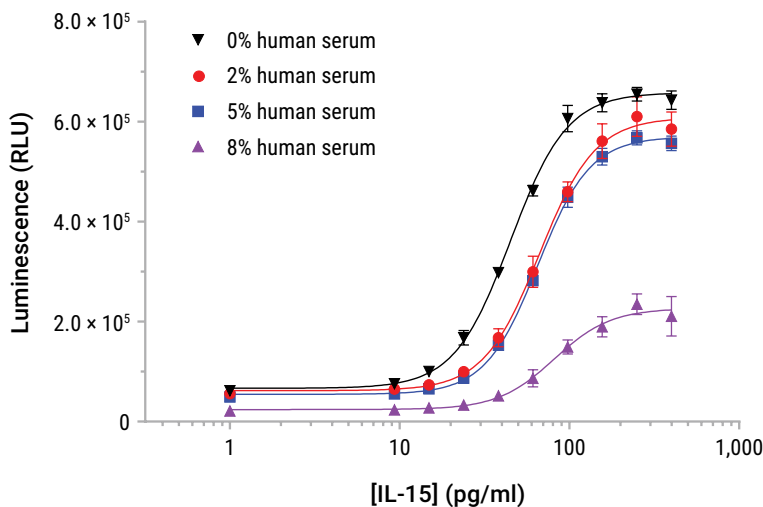


| | 96 | 384 |
|------------------|-------|-------|
| EC ₅₀ | 51.70 | 53.28 |

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Figure 6. The IL-15 Bioassay is amenable to 384-well plate format. The IL-15 Bioassay was tested in 96- and 384-well formats. IL-15 Bioassay Cells were prepared and dispensed as 50 μ l (96-well) or 12.5 μ l (384-well) volumes. Serial 1.7-fold dilutions of recombinant human IL-15 were prepared and added to cells (25 μ l/well in the 96-well format; 6.2 μ l/well in the 384-well format). After 6 hours of stimulation with recombinant IL-15, Bio-Glo™ Reagent was added (75 μ l/well in the 96-well format; 18.7 μ l/well in the 384-well format), and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. Data were generated using thaw-and-use cells. The IL-15 EC₅₀ was approximately 52pg/ml for both plate formats.

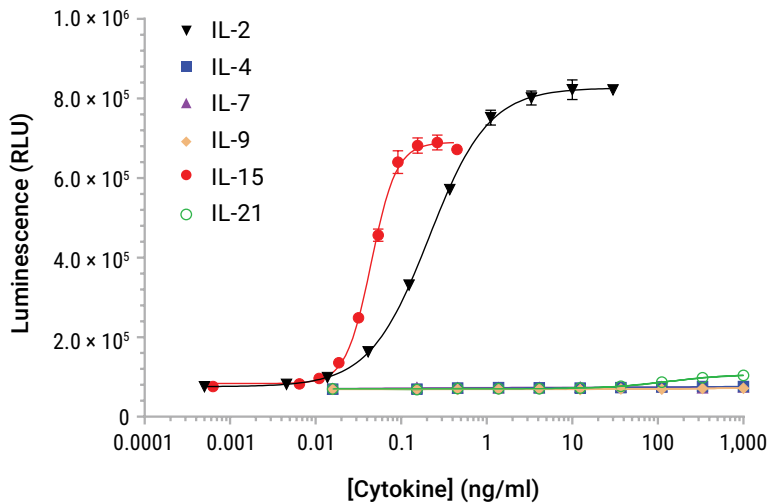
1. Description (continued)



| | 0% | 2% | 5% | 8% |
|------------------|-------|-------|-------|-------|
| EC ₅₀ | 45.06 | 66.58 | 65.09 | 81.52 |

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Figure 7. The IL-15 Bioassay tolerates up to 5% human serum. IL-15 Bioassay Cells were tested with a dose-response of recombinant IL-15 in the absence or presence of increasing concentrations of pooled normal human serum, resulting in final assay concentrations of human serum (0–8%). Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.



| | IL-2 | IL-15 |
|------------------|--------|---------|
| EC ₅₀ | 0.2118 | 0.04461 |

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Figure 8. IL-15 Bioassay cytokine specificity. IL-15 Bioassay Cells were tested with a panel of related type-1 cytokines (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21). Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. Data were generated using thaw-and-use cells.



2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|-----------------------|---------------|---------------|
| IL-15 Bioassay | 1 each | JA2011 |

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 IL-15 Bioassay Cells, 1.2×10^7 cells/ml (0.8ml per vial)
- 36ml RPMI 1640 Medium
- 4ml Fetal Bovine Serum
- 1 vial Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 10ml Bio-Glo™ Luciferase Assay Buffer

| PRODUCT | SIZE | CAT.# |
|--------------------------|---------------|---------------|
| IL-15 Bioassay 5X | 1 each | JA2015 |

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 IL-15 Bioassay Cells, 1.2×10^7 cells/ml (0.8ml per vial)
- 5 x 36ml RPMI 1640 Medium
- 5 x 4ml Fetal Bovine Serum
- 5 vials Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 5 x 10ml Bio-Glo™ Luciferase Assay Buffer

Note: IL-15 Bioassay components are shipped separately because of differing temperature requirements. The IL-15 Bioassay Cells are shipped on dry ice. The Bio-Glo™ Luciferase Assay System 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 $+2^{\circ}\text{C}$ to $+10^{\circ}\text{C}$ protected from light.

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 web site such as Certificate of Analysis.

The IL-15 Bioassay is intended for use with user-provided biologics designed to activate or inhibit the IL-15 signaling pathway. The protocol and components described in Section 4 were established using research-grade recombinant human IL-15. You may need to adjust the parameters provided here and optimize assay conditions for other biologic samples.

The IL-15 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 luminometer. An integration time of 0.5 seconds/well was used for all readings. The bioassay is compatible with most other plate-reading luminometers; however, relative luminescence unit readings may vary due to the sensitivity and settings of each instrument. The use of different instruments should not affect the measured relative potency of test samples.

3.A. Materials to Be Supplied by the User

Reagents

- user-defined biologics samples
- **optional:** recombinant human IL-15 (e.g., PeproTech Cat.# 200-15)

Supplies and Equipment

- white, flat-bottom 96-well assay plates (e.g., Corning[®] Cat.# 3917)
- sterile clear 96-well plate with lid (e.g., Corning[®] Cat.# 3896 or Falcon Cat.# 353077) for preparing sample dilutions
- pipettes (single-channel and 12-channel)
- sterile 15ml and 50ml conical tubes
- sterile reagent reservoirs (e.g., Corning[®] Cat.# 4870)
- 37°C, 5% CO₂ humidified incubator
- 37°C water bath
- plate reader that measures glow luminescence or luminometer (e.g., GloMax[®] Discover System)



4. Assay Protocol

The IL-15 Bioassay can be used to test IL-15 type biological samples such as recombinant human IL-15. This protocol illustrates the use of the IL-15 Bioassay to examine two test samples against a reference sample in a single assay run. Each test and reference sample 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.

Notes:

1. When preparing test and reference samples, 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 0–600pg/ml of recombinant human IL-15 (PeproTech Cat. #200-15) as a sample range, with serial 1.8-fold dilutions to achieve full dose curves as 10-point series. Concentration ranges and dilution schemes may need to be optimized for your samples.
2. When diluted as directed, each kit containing medium, serum, and 1 vial of IL-15 Bioassay Cells is sufficient for 120 wells (two 96-well plates using inner-60 format). The thaw-and-use cells are for single use only and cannot be cultured or refrozen for second use. Please plan your experiments accordingly to optimize the use of the thaw-and-use cells.

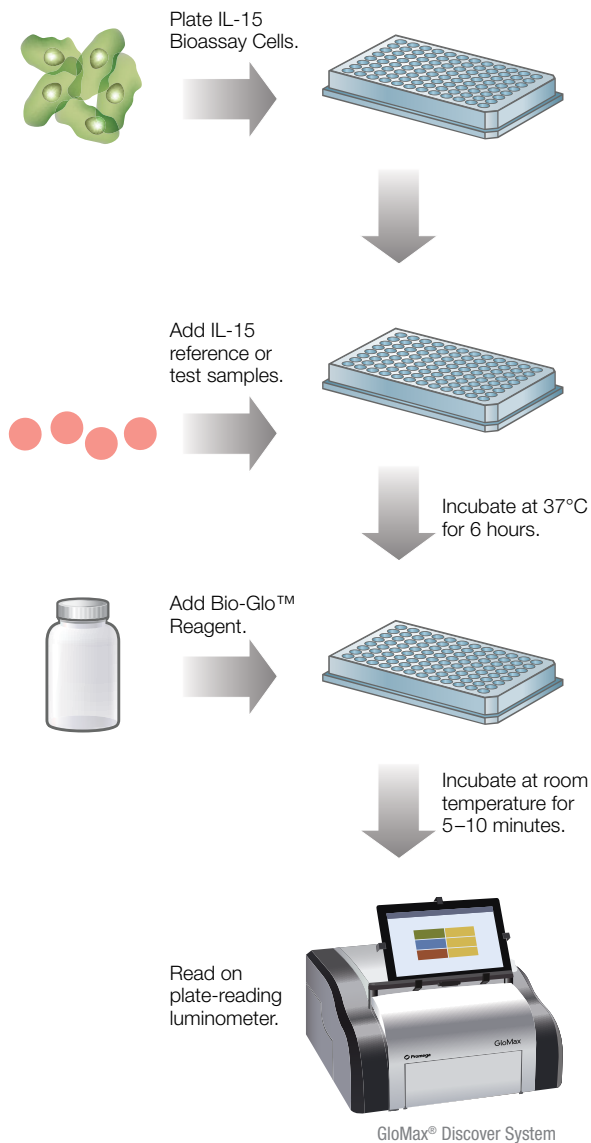


Figure 9. IL-15 Bioassay schematic protocol.

4.A. Plate Layout Design

For the protocol described here, use the plate layout illustrated in Figure 10 as a guide. The protocol describes serial replicate dilutions (n = 3) of test and reference samples 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 drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | B | Reference Replicate 1 |
| C | B | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | B | Test Replicate 1 |
| D | B | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | B | Reference Replicate 2 |
| E | B | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | B | Test Replicate 2 |
| F | B | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | B | Reference Replicate 3 |
| G | B | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | B | Test Replicate 3 |
| H | B | B | B | B | B | B | B | B | B | B | B | B | Assay Buffer (B) |

Figure 10. Example plate layout. This suggested layout shows nonclustered locations for three replicates of each test and reference sample dilution series (dilu1–dilu9) and wells containing assay buffer (denoted by “B”) alone.

4.B. Preparing Reagents for the Assay Day

1. **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 in a refrigerator 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.

On the day of the assay, thaw the appropriate amount of reconstituted Bio-Glo™ Reagent in a room temperature water bath for at least 1–2 hours before use. After reconstitution, the Bio-Glo™ Reagent can be stored at room temperature with ~18% loss in luminescence after 24 hours or at 4°C with ~12% loss of luminescence after 5 days.

2. **Assay Buffer:** Ensure that an appropriate amount of assay buffer is prepared for the assay. Thaw the fetal bovine serum (FBS) overnight at 4°C, or in a 37°C water bath, taking care not to overheat it. To make 40ml of assay buffer, add 4ml of FBS to 36ml of RPMI 1640 Medium to yield 90% RPMI 1640/10% FBS (see Section 7.A). Mix well and warm to 37°C prior to use. For reference, 40ml of assay buffer is typically sufficient for 120 wells in a 96-well assay format using the inner 60 wells.

4.C. Preparing and Plating IL-15 Bioassay Cells

The thaw-and-use IL-15 Bioassay Cells included in this kit are sensitive; carefully 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. We recommend that you thaw and dilute a maximum of two vials of thaw-and-use cells at a time.



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

1. Remove one vial of IL-15 Bioassay Cells from storage at –140°C and transfer to the bench on dry ice.
2. Add 7.2ml of prewarmed (37°C) assay buffer to a 15ml conical tube.
3. Warm the cells in a 37°C water bath until just thawed (about 2 minutes). While thawing, gently agitate and visually inspect. Try not to submerge the vial completely. Do not invert.
4. Gently mix the cell suspension by pipetting, then transfer 0.8ml of the cells to the 15ml conical tube containing 7.2ml of assay buffer. Mix well by gently pipetting or inverting 5 times.
5. Transfer the cell suspension to a sterile reagent reservoir.
6. Using a multichannel pipette, immediately dispense 50µl of the cell suspension to each of the inner 60 wells of two 96-well assay plates. Optimal results depend on gently keeping the cells evenly resuspended during the plating process.
7. Add 75µl/well of warm assay buffer to outer 36 wells of each plate.
8. Cover each assay plate with a lid and incubate in a 37°C, 5% CO₂ humidified incubator while preparing samples and dilutions.



4.D. Preparing Serial Dilutions

Serial dilutions should be prepared after plating the IL-15 Bioassay Cells, on the day of the assay.

The instructions described here are for preparation of a single stock of 1.8-fold serial dilutions of a single sample for analysis in triplicate (120µl 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 1.8-fold serial dilutions, you will need a total of 540µl of a reference sample at 3X the highest concentration in your dose-response curve. You will need 270µl of each test sample at 3X the highest concentration in each of the test sample dose-response curves. For other dilution schemes, adjust the volumes accordingly.

Notes on recommended starting concentration of reference samples:

For IL-15 stimulation using recombinant human IL-15 as your reference sample (PeproTech IL-15, Cat.# 200-15), we recommend starting with a 3X concentration of 1.8ng/ml and performing serial 1.8-fold dilutions. When using other reference sources of IL-15, the starting concentration may need to be adjusted.

1. To a sterile clear 96-well plate, add 270µl of reference sample starting dilution (dilu1, 3X final concentration) to wells A11 and B11 (see Figure 11).
2. Add 270µl of test samples 1 and 2 starting dilution (dilu1, 3X final concentration) to wells C11 and D11, respectively.
3. Add 120µl of assay buffer to other wells in these four rows, from column 10 to column 2.
4. Transfer 150µl of the sample starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent 1.8-fold serial dilutions across the columns from right to left until you reach column 3. Remove 150µl from column 3 so all wells have 120µl volume. Do not dilute into column 2.
6. Cover the plate with a lid and proceed to Section 4.E.

| Recommended Plate Layout for Sample Dilutions Prepared from a Single Sample Stock | | | | | | | | | | | | | |
|---|---|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|------------------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| A | | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | | Reference sample |
| B | | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | | Reference sample |
| C | | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | | Test sample 1 |
| D | | no drug | dilu9 | dilu8 | dilu7 | dilu6 | dilu5 | dilu4 | dilu3 | dilu2 | dilu1 | | Test sample 2 |
| E | | | | | | | | | | | | | |
| F | | | | | | | | | | | | | |
| G | | | | | | | | | | | | | |
| H | | | | | | | | | | | | | |

Figure 11. Example plate layout showing reference and test sample serial dilutions. Wells A2, B2, C2 and D2 contain 120µl of assay buffer without sample as a negative control.

4.E. IL-15 Stimulation Assay

1. Using a multichannel pipette, dispense 25µl of each sample to the 50µl of preplated cells according to the plate layout in Figure 10.
2. Cover each assay plate with a lid and incubate in a 37°C, 5% CO₂ humidified incubator for 6 hours.
3. After a 6-hour incubation, proceed to Section 4.F.



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, remove the plate lid, and equilibrate to ambient temperature for 10–15 minutes.
2. Using a multichannel pipette, add 75µl of Bio-Glo™ Reagent to the inner 60 wells of the assay plates, taking care not to create bubbles.
3. Add 75µl 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 RLU values but should not significantly change the EC₅₀ value and fold induction.
5. Measure luminescence using a luminometer or luminescence plate reader.

4.G. Data Analysis

1. Measure plate background by calculating the average RLU from wells B1, C1 and D1.

2. Calculate fold induction =
$$\frac{\text{RLU (sample-background)}}{\text{RLU (no drug control-background)}}$$

Note: When calculating fold induction, if the no drug control sample RLUs are at least 100X the plate background RLUs, there is no need to subtract plate background from sample RLU.

3. Graph data as RLU versus Log₁₀ [sample] and fold induction versus Log₁₀ [sample]. Fit curves and determine the EC₅₀ value of IL-15 response using appropriate curve fitting software (such as GraphPad Prism®).

5. 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 | Causes and Comments |
|---|--|
| Low luminescence measurements (RLU readout) | <p>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.</p> <p>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.</p> <p>Low activity of Bio-Glo™ Reagent leads to low RLU. Store and handle the Bio-Glo™ Reagent according to the instructions.</p> |
| Variability in assay performance | <p>Ensure that incubation times are consistent between assays.</p> <p>Ensure serial dilutions of samples are consistently prepared.</p> |
| Weak assay response (low fold induction) | <p>Optimize the concentration range of your test sample(s) to achieve a full dose response with complete upper and lower asymptotes.</p> <p>Ensure the assay incubation period is 6 hours and not overnight. Overnight assay incubation does not work for this assay.</p> <p>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.</p> |

6. References

1. Burton, J.D. *et al.* (1994) A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc. Natl. Acad. Sci.* **91**, 4935–9.
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7. Appendix

7.A. Composition of Buffers and Solutions

assay buffer

90% RPMI 1640 Medium

10% fetal bovine serum

Prepare day of the assay and warm to 37°C before use.

7.B. Related Products

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.

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 |
| Fc γ R1Ia-H ADCP Reporter Bioassay, Complete Kit** | 1 each | G9901 |
| Fc γ R1Ia-H ADCP Reporter Bioassay, Core Kit** | 1 each | G9991 |
| Mouse Fc γ RIV ADCC Bioassay, Complete Kit | 1 each | M1201 |
| Mouse Fc γ RIV 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.

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.



7.B. Related Products (continued)

Immune Checkpoint Bioassays

| Product | Size | Cat.# |
|---------------------------------|--------|--------|
| 4-1BB Bioassay | 1 each | JA2351 |
| CD28 Bioassay | 1 each | JA6701 |
| CD28 Blockade Bioassay | 1 each | JA6101 |
| CD40 Bioassay | 1 each | JA2151 |
| CTLA-4 Blockade Bioassay | 1 each | JA3001 |
| GITR Bioassay | 1 each | JA2291 |
| ICOS Bioassay | 1 each | JA6801 |
| ICOS Blockade Bioassay | 1 each | JA6001 |
| LAG-3/MHCII Blockade Bioassay | 1 each | JA1111 |
| OX40 Bioassay | 1 each | JA2191 |
| PD-1/PD-L1 Blockade Bioassay | 1 each | J1250 |
| PD-1+TIGIT Combination Bioassay | 1 each | J2211 |
| PD-L1 Negative Cells | 1 each | J1191 |
| TIGIT/CD155 Blockade Bioassay | 1 each | J2201 |

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

T Cell Activation Bioassays

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

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

Control Antibodies and Proteins

| Product | Size | Cat. # |
|-------------------------|-------|--------|
| Control Ab, Anti-4-1BB | 50µg | K1161 |
| Control Ab, Anti-CD-20 | 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 ligand | 10µg | J2371 |

Detection Reagent

| Product | Size | Cat. # |
|-------------------------------------|---------|--------|
| Bio-Glo™ Luciferase Assay System | 10ml | G7941 |
| | 100ml | G7940 |
| Bio-Glo-NL™ Luciferase Assay System | 10ml | J3081 |
| | 100ml | J3082 |
| | 1,000ml | J3083 |

Not for Medical Diagnostic Use.

Luminometers

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

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Note: Additional Cytokine and Growth Factor Bioassays are available. 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



8. Summary of Changes

The following changes were made in the 6/23 revision of this document:

1. Updated the RPMI 1640 Medium volume and storage temperature ranges in Section 2.
2. Revised the disclaimers.

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^(b)Product cannot be used for proficiency testing.

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

^(d)U.S. Pat. No. 10,077,244 and other patents.

^(e)Patent Pending.

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