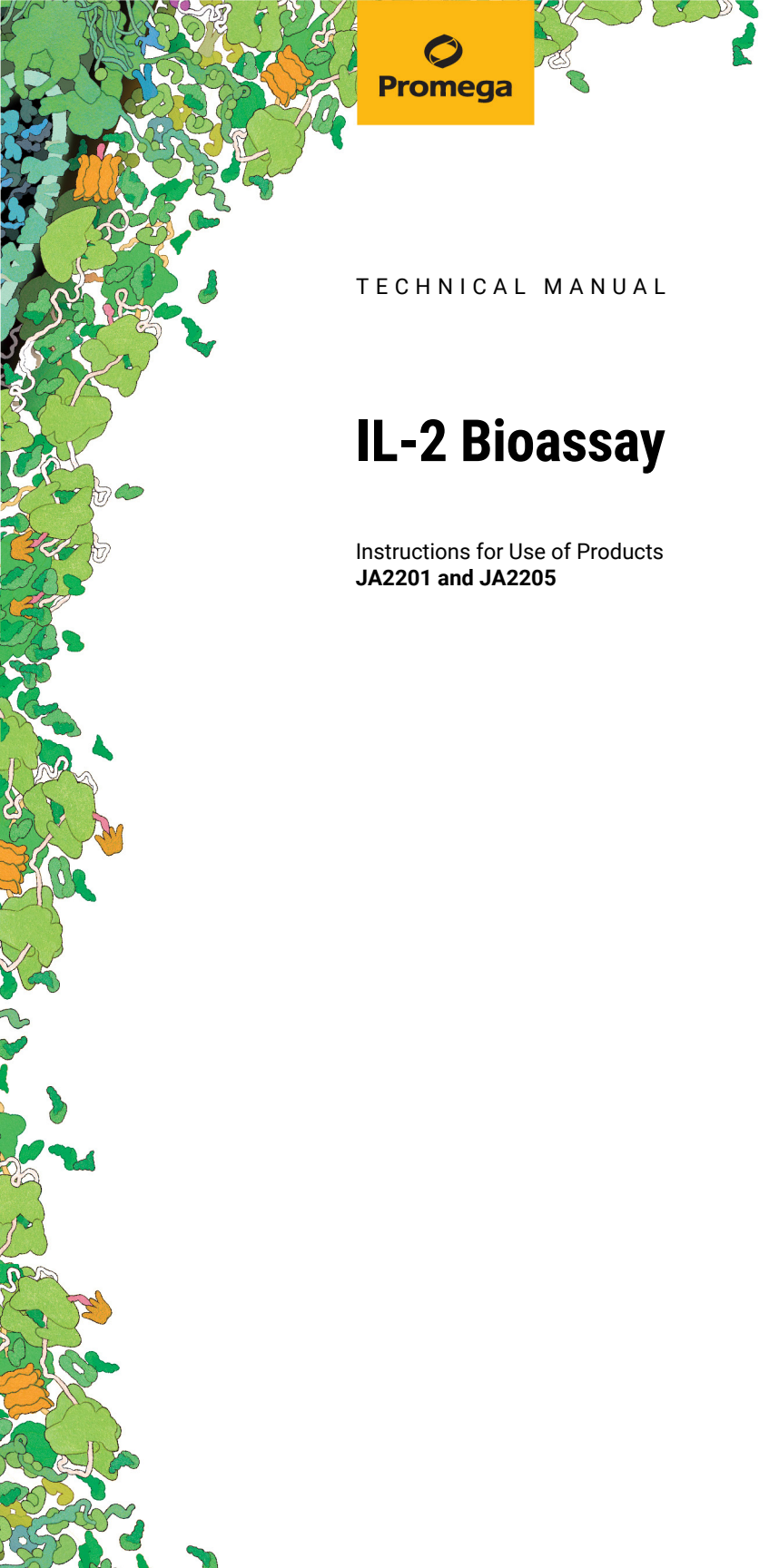


TECHNICAL MANUAL

IL-2 Bioassay

Instructions for Use of Products
JA2201 and JA2205

Revised 5/26
TM578

IL-2 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

Promega

1. Description

Interleukin-2 (IL-2), originally described as "T cell growth factor" in 1976, is a small 15.5kDa monomer secreted by a variety of cell types including CD4+ and CD8+ T cells, natural killer (NK) cells and activated dendritic cells (1,2). IL-2 has pleiotropic effects on the immune system. It plays a critical role in the generation, maintenance and expansion of CD4+ regulatory T cells, promotes the cytotoxic activity of NK and CD8+ cells and governs homeostasis through the elimination of harmful autoreactive T cells via activation-induced cell death.

Aldesleukin, a biologic based on human IL-2 with minor sequence modifications, is FDA approved for the treatment of both metastatic renal cell carcinoma (1992) and metastatic melanoma (1998) as a monotherapy (3). Its antitumor efficacy is achieved by increasing proliferation of NK cells, lymphokine-activated killer cells and other cytotoxic cells. Though initially promising, its current clinical usage is limited due to a variety of severe side effects at high dosages. Modern therapeutic strategies involving IL-2 attempt to overcome the limitations of aldesleukin, including: combination therapies (cell-based immunotherapy, chemotherapeutic agents, peptide vaccines and checkpoint inhibitors) and formats with sustained or targeted activity (immunocytokine, immunocomplex, PEGylation and protease activation; 4).

The IL-2 receptor (IL-2R) is a type 1 cytokine receptor consisting of 3 subunits: IL-2Rα (CD25), IL-2Rβ (CD122) and IL-2Rγ (CD132). IL-2Rγ is also known as the γ common chain (γc) and is found in receptors for IL-4, IL-7, IL-9, IL-15 and IL-21. The IL-2 trimeric receptor form consists of all 3 subunits and has the highest affinity for IL-2 (K_d ~10^{-11}M). A heterodimeric form, containing only IL-2Rβ and γc, has medium affinity (K_d ~10^{-9}M), while the IL-2Rα alone has low affinity (K_d ~10^{-8}M) and lacks a cytoplasmic signaling domain (5). Various forms of the IL-2 receptors are located on T cells, B cells and NK cells. The highly expressed IL-2Rα subunit is responsible for the initial binding and conformation change of IL-2 prior to its association with the β and γ subunits and subsequent intracellular signaling.

When IL-2 is bound to either the dimeric or trimeric receptor forms, multiple signaling pathways are activated. JAK1 and JAK3 tyrosine kinases are recruited and activated at the receptor cytoplasmic domains. These kinases, in turn, activate the STAT, PI3-AKT and MAPK signaling pathways, which then mediate proliferation, survival, activation and differentiation in a multitude of immune cell types (3).

The IL-2 Bioassay[a–d] (Cat.# JA2201, JA2205) is a bioluminescent cell-based assay designed to measure IL-2 stimulation or inhibition. The IL-2 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-2 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-2 Bioassay, Propagation Model, Cat.# J2952).

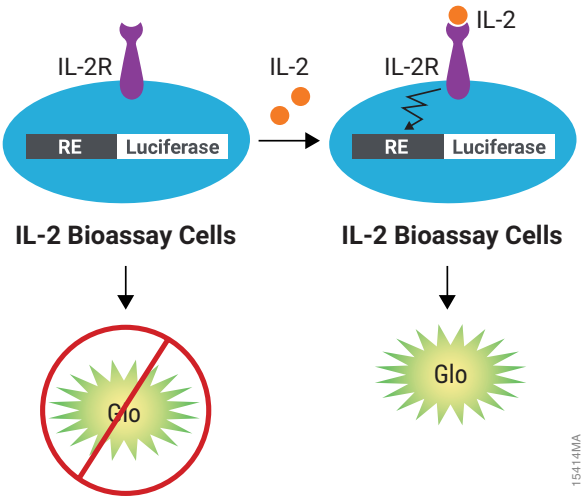


Figure 1. Representation of the IL-2 Bioassay. The IL-2 Bioassay consists of a genetically engineered cell line, IL-2 Bioassay Cells. When IL-2 binds to its receptor (IL-2R), receptor-mediated pathway signaling induces luminescence that can be detected upon addition of Bio-Glo™ Reagent and quantified with a luminometer. In the absence of IL-2, no signaling occurs downstream of IL-2R and no luminescent signal is generated.

1. Description (continued)

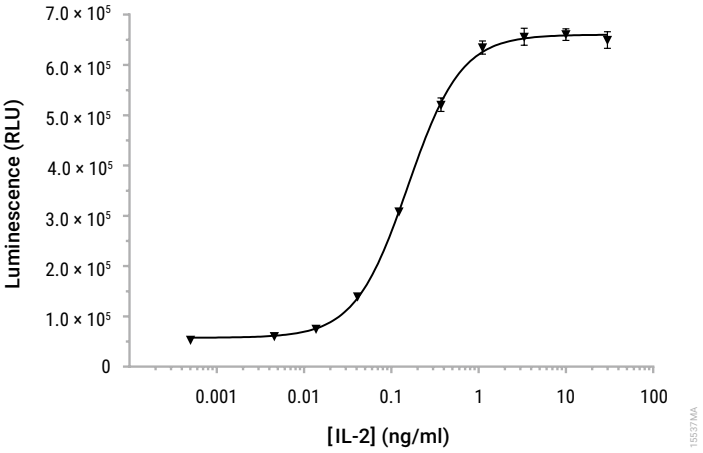


Figure 2. The IL-2 Bioassay is responsive to recombinant IL-2. IL-2 Bioassay Cells were prepared as described in this protocol and incubated with serial dilutions of recombinant IL-2. After a 6-hour incubation, Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. The EC_{50} was 0.155ng/ml, with a fold induction of 12. Data were generated using thaw-and-use cells.

Table 1. The IL-2 Bioassay Shows Precision, Accuracy and Linearity.

Parameter	Results	
Accuracy	% Expected Relative Potency	% Recovery
	50	103.9
	70	99.8
	140	100.5
	200	101.6
Repeatability (% CV)	100% (Reference)	4.32
Intermediate Precision (% CV)		6.88
Linearity (r^2)		0.999
Linearity (y = mx + b)		y = 1.01x + 0.09

A 50–200% theoretical potency series of aldesleukin (therapeutic IL-2) 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.

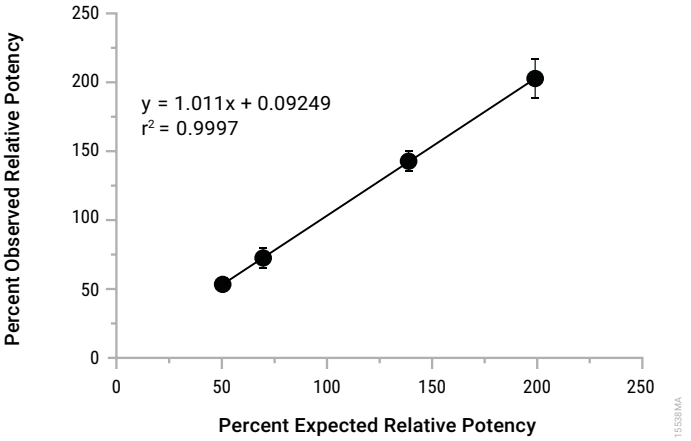


Figure 3. The IL-2 Bioassay shows precision, accuracy and linearity. A 50–200% theoretical potency series of aldesleukin (therapeutic IL-2) was analyzed in triplicate in three independent experiments performed on three days by two analysts using the IL-2 Bioassay (for a total of six independent experiments). 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.

1. Description (continued)

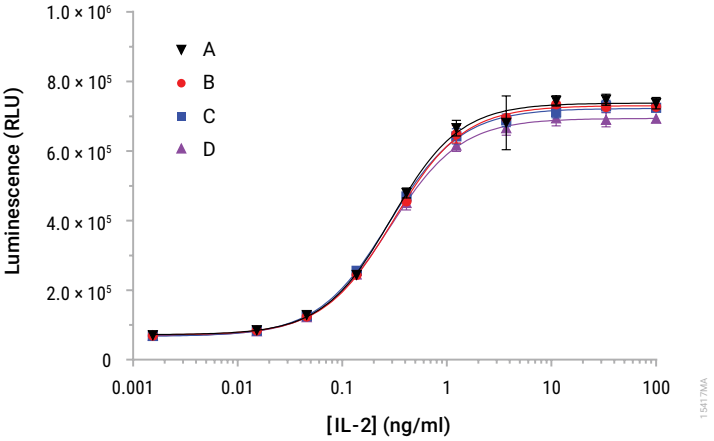


Figure 4. The IL-2 Bioassay demonstrates repeatability. Four separate dilution series of aldesleukin (therapeutic IL-2) were analyzed on four individual assay plates using the IL-2 Bioassay. Bio-Glo™ Reagent was added, and luminescence was quantified using the GloMax® Discover System. Data were fitted to a 4PL curve using GraphPad Prism® software. The EC_{50} values were 0.306, 0.309, 0.284 and 0.287ng/ml on the four assay plates. Data were generated using thaw-and-use cells.

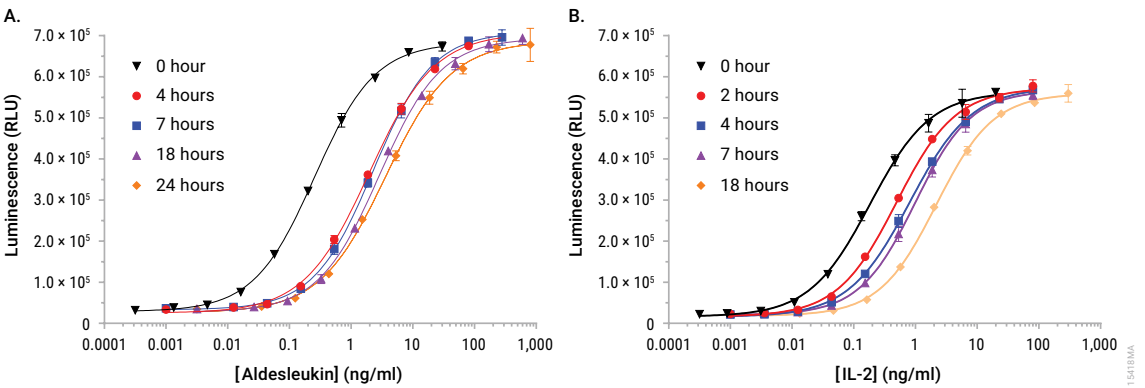


Figure 5. The IL-2 Bioassay indicates stability. Proleukin® (aldesleukin) (**Panel A**, Clinigen, 1.1mg/ml undiluted) or a research grade equivalent (**Panel B**, Miltenyi Cat.# 130-097-742, 20µg/ml with BSA carrier) was heat stressed at 53°C prior to being tested in the IL-2 Bioassay. Bio-Glo™ Reagent 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.

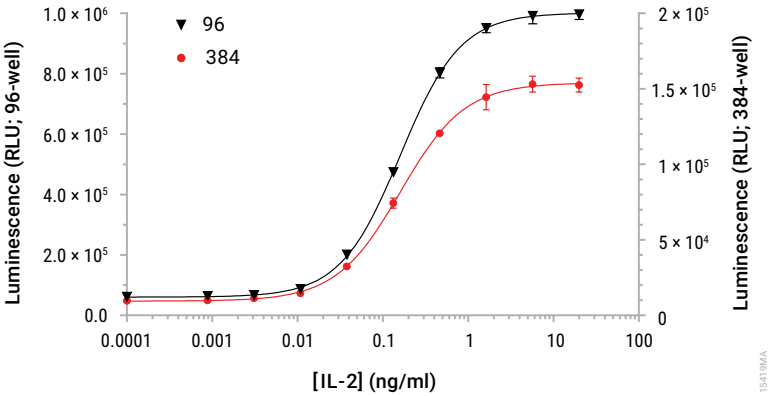
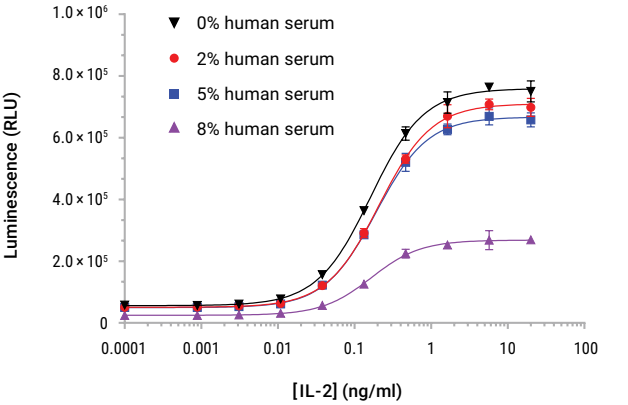


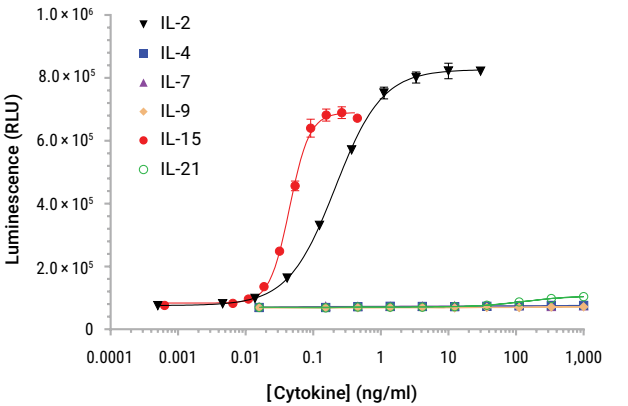
Figure 6. The IL-2 Bioassay is amenable to 384-well plate format. The IL-2 Bioassay was tested in 96- and 384-well formats. IL-2 Bioassay Cells were prepared and dispensed as 50µl (96-well) or 12.5µl (384-well) volumes. Serial dilutions of recombinant IL-2 were prepared and added to cells (25µl/well for 96-well plates; 6.2µl/well for 384-well plates). After 6 hours of stimulation with recombinant IL-2, Bio-Glo™ Reagent was added (75µl/well for 96-well plates; 18.7µl/well for 384-well plates), 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-2 EC_{50} was approximately 0.16ng/ml for both plate formats.

1. Description (continued)



	0%	2%	5%	8%
EC_{50}	0.1622	0.2085	0.1913	0.1659

Figure 7. The IL-2 Bioassay tolerates up to 5% human serum. IL-2 Bioassay Cells were tested with a dose-response of recombinant IL-2 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

Figure 8. IL-2 Bioassay cytokine specificity. IL-2 Bioassay Cells were tested with a panel of related type 1 cytokines (IL-2, IL-15, IL-4, IL-7, IL-9 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-2 Bioassay	**1 each**	**JA2201**

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-2 Bioassay Cells, 1.2×10^7 cells/ml (0.80ml 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-2 Bioassay 5X	**1 each**	**JA2205**

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-2 Bioassay Cells, 1.2×10^7 cells/ml (0.80ml per vial)
- 5 × 36ml RPMI 1640 Medium
- 5 × 4ml Fetal Bovine Serum
- 5 vials Bio-Glo™ Luciferase Assay Substrate (lyophilized)
- 5 × 10ml Bio-Glo™ Luciferase Assay Buffer

Note: IL-2 Bioassay components are shipped separately because of differing temperature requirements. The IL-2 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°C to +10°C protected from light.

Promega

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 the Certificate of Analysis.

The IL-2 Bioassay is intended for use with user-provided biologics designed to activate or inhibit the IL-2 signaling pathway. The protocol and components described in Section 4 were established using research-grade recombinant human IL-2. You may need to adjust the parameters provided here and optimize assay conditions for other biologic samples.

The IL-2 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-2 (e.g., PeproTech Cat.# 200-02)

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 dilutions
- pipettes (single-channel and 12-channel)
- 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
- plate reader that measures glow luminescence or luminometer (e.g., GloMax® Discover System)

4. Assay Protocol

The IL-2 Bioassay can be used to test IL-2 type biological samples such as recombinant human IL-2 and aldesleukin (therapeutic IL-2). This protocol illustrates the use of the IL-2 Bioassay to examine two test samples against a reference sample in a single assay run (Figure 9). 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 (Figure 10). Other experimental and plate layouts are possible but may require further optimization.

Notes:

a. 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 0–30ng/ml of recombinant IL-2 (Peprotech Cat.# 200-02) as a sample range, with serial threefold dilutions to achieve full dose curves as 10-point series. Concentration ranges and dilution schemes may need to be optimized for your samples.

b. When diluted as directed, each kit containing medium, serum and one vial of IL-2 Bioassay Cells is sufficient for 120 wells (two 96-well plates using the 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.

4. Assay Protocol (continued)

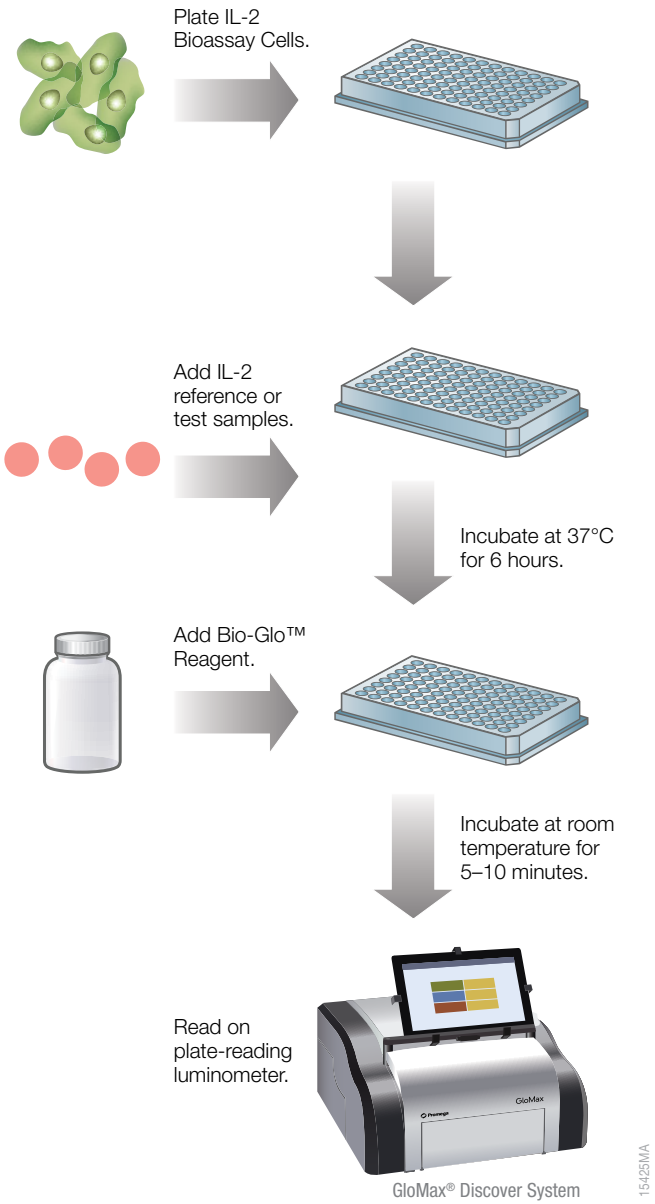


Figure 9. IL-2 Bioassay schematic protocol.

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 ten-point dose-response curves for each plate.

Recommended Plate Layout Design													
	1	2	3	4	5	6	7	8	9	10	11	12	
A	B	B	B	B	B	B	B	B	B	B	B	B	Assay Buffer (B)
B	B	no 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

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. The Bio-Glo™ Reagent can be stored at room temperature after reconstitution 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 plate wells.

4.C. Preparing and Plating IL-2 Bioassay Cells

The thaw-and-use IL-2 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-2 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 and transfer 0.8ml of the cells to the 15ml conical tube containing 7.2ml of assay buffer. Mix well by gently pipetting or inverting five 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 (see Figure 10). 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 lid and place at 37°C, 5% CO_2 while preparing samples and dilutions.

4.D. Preparing Serial Dilutions

Serial dilutions should be prepared after plating the IL-2 Bioassay Cells, on the day of assay.

The instructions described here are for preparation of a single stock of threefold 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 threefold serial dilutions, you will need a total of 360µl of a reference sample at 3X the highest concentration in your dose-response curve. You will need 180µl 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-2 stimulation using recombinant human IL-2 as your reference sample (PeproTech IL-2 Cat.# 200-02), we recommend starting with a 3X concentration of 90ng/ml and performing serial threefold dilutions. When using other reference sources of IL-2, the starting concentration may need to be adjusted.

1. To a sterile clear 96-well plate, add 180µl of reference sample starting dilution (dilu1, 3X final concentration) to wells A11 and B11 (see Figure 11).
2. Add 180µl 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 60µl of the sample starting dilutions from column 11 into column 10. Mix well by pipetting. Avoid creating bubbles.
5. Repeat equivalent threefold serial dilutions across the columns from right to left until you reach column 3. Remove 60µl 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.

4.D. Preparing Serial Dilutions (continued)

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. Note: Wells A2, B2, C2 and D2 contain 120µl of assay buffer without sample as a negative control.

4.E. IL-2 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% CO_2 humidified incubator for 6 hours.
3. After the 6-hour incubation is over, 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 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 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. 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 RLU is at least 100X the plate background RLU, there is no need to subtract plate background from sample RLU.
3. Graph data as RLU versus Log_{10}[sample] and fold induction versus Log_{10}[sample]. Fit curves and determine the EC_{50} value of IL-2 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**. 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.
	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.
Assay performance is variable	Ensure that incubation times are consistent between assays.
	Ensure that sample serial dilutions are consistently prepared.
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.
	Ensure that the assay incubation period is 6 hours and not overnight. Overnight assay incubation does not work for this assay.
	If untreated control RLU is less than 100X above plate reader background RLU, subtract plate reader background RLU from all samples prior to calculating fold induction.

6. References

1. Morgan, D.A. Ruscetti, F.W. and Gallo, R. (1976) Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* **193**, 1007–8.
2. Ruscetti, F.W. Morgan, D.A. and Gallo, R.C. (1977) Functional and morphologic characterization of human T cells continuously grown in vitro. *J. Immunol.* **119**, 131–8.
3. Jiang, T., Zhou, C. and Ren, S. (2016) Role of IL-2 in cancer immunotherapy. *Oncoimmunology* **5**, e1163462.
4. Boyman, O. and Arenas-Ramirez, N. (2019) Development of a novel class of interleukin-2 immunotherapies for metastatic cancer. *Swiss Med. Wkly*. **149**, w14697.
5. Spolski, R. Li, P. and Leonard, W.J. (2018) Biology and regulation of IL-2: from molecular mechanisms to human therapy. *Nature. Rev. Immunol.* **18**, 648–59.

7. Appendix

7.A. Composition of Buffers and Solutions

assay buffer

90% RPMI 1640
10% FBS

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

8. Summary of Changes

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

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

Promega

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

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

[b]U.S. Pat. No. 8,008,006.

[c]U.S. Pat. No. 10,077,244 and other patents.

[d]Patent Pending.