QuantiGlo®

Human IL-15 Chemiluminescent Immunoassay

Catalog Number Q1500B

For the quantitative determination of human interleukin 15 (IL-15) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Interleukin 15 (IL-15) is a novel cytokine that shares many biological properties with IL-2. It was originally identified in media conditioned by a monkey kidney epithelial cell line (CVI/EBNA) based on its mitogenic activity on the mouse T cell line, CTLL-2 (1). IL-15 was independently discovered as a cytokine produced by a human adult T-cell leukemia cell line (HuT-102) that stimulated T cell proliferation and was designated IL-T (2). Descriptions of the discovery and properties of IL-15 can be found in several reviews (3 - 5).

Human, simian and mouse IL-15 cDNAs have been isolated and characterized (1, 6, 7). The IL-15 cDNA clones from all three species encode a 162 amino acid (aa) residue precursor protein that contains a 48 aa leader sequence and a 114 aa mature IL-15 subunit. In humans, an alternative splice form has also been identified. This isoform has an identical mature sequence, but a shorter signal sequence. Notably, the shorter pre-peptide is not secreted and may represent a functional intracellular molecule (8, 9). In addition, a membrane-bound form of IL-15 has also been reported, which may be an important physiological form of the molecule (10). Human IL-15 shares approximately 97% and 73% aa sequence identity with simian and mouse IL-15, respectively. Both human and simian IL-15 are active on mouse cells.

High-affinity cell surface receptors for IL-15 have been detected on a variety of cells including T cells, B cells, and NK cells as well as on non-lymphoid cells (11, 12). The IL-15 receptor is composed of three molecules; an IL-15 specific α-chain, plus a β-chain and γ-chain that are shared by the receptor system for IL-2. The α-chain is 237 aa in length and binds IL-15 with high affinity. It shows 54% aa sequence identity with mouse IL-15 Rα (13, 14) and exists in multiple alternatively spliced forms (14, 15). Although the IL-15 receptor is composed of 3 chains, IL-15 will signal through a $\beta\gamma$ dimer (14) and the α-chain itself may have multiple functions (16).

IL-15 has biological activities similar to IL-2 and has been shown to stimulate the growth of natural killer cells, activated peripheral blood T lymphocytes (1, 11, 12), tumor infiltrating lymphocytes (TILs) (17), and B cells (18). In addition, IL-15 has also been shown to be a chemoattractant for human blood T lymphocytes (19) and to induce both lymphokine-activated killer (LAK) activity in NK cells and the generation of cytolytic effector cells. IL-15 apparently also has effects on cells not involved in immune responses. Skeletal muscle cells express IL-15 and IL-15 R mRNAs and respond to addition of IL-15 (20).

The QuantiGlo Human IL-15 Chemiluminescent Immunoassay is a 5.5 hour solid phase ELISA designed to measure human IL-15 levels in cell culture supernates, serum, and plasma. It contains recombinant human IL-15 expressed in *E. coli* and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-15 showed linear curves that were parallel to the standard curves obtained using the recombinant QuantiGlo kit standards. These results indicate that the QuantiGlo immunoassay can be used to determine relative mass values for natural human IL-15.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-15 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-15 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for IL-15 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells and light is produced in proportion to the amount of IL-15 bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted.

TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Variation in pipetting technique, washing technique, luminometers, incubation time or temperature can cause variations in binding.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested in the QuantiGlo Immunoassay, the possibility of interference cannot be excluded.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Relative light units (RLUs) may differ among luminometers. The QuantiGlo Human IL-15 Immunoassay was optimized using a DYNEX TECHNOLOGIES MLX™ luminometer. Other instruments may require settings to be adjusted.
- Relative light units may vary within the 15 minute reading window.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low, beginning with the high standard in wells A_1 and A_2.

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MATERIALS PROVIDED

IL-15 Microplate (Part 893579) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-15.

IL-15 Conjugate (Part 893629) - 21 mL of mouse monoclonal antibody against IL-15 conjugated to horseradish peroxidase with preservatives.

IL-15 Standard (Part 890919) - 15 ng of recombinant human IL-15 in a buffered protein base with preservatives; lyophilized.

Assay Diluent RD1-19 (Part 895467) - 11 mL of a buffered protein base with preservatives.

Calibrator Diluent RD5R (Part 895190) - 21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*

Calibrator Diluent RD6-9 (Part 895423) - 21 mL of a buffered protein base with preservatives. *For serum/plasma samples.*

Wash Buffer Concentrate (Part 895222) - 100 mL of a 10-fold concentrated solution of buffered surfactant with preservatives.

Glo Reagent A (Part 895868) - 4 mL of stabilized enhanced luminol.

Glo Reagent B (Part 895869) - 8 mL of stabilized hydrogen peroxide.

Plate Covers - 4 adhesive strips.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Calibrator Diluent RD5R</td></tr>
<tr><td>Calibrator Diluent RD6-9</td></tr>
<tr><td>Assay Diluent RD1-19</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Glo Reagent A</td></tr>
<tr><td>Unmixed Glo Reagent B</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- DYNEX TECHNOLOGIES MLX luminometer set with the following parameters: 1.0 minute lag time; 0.5 sec/well read time; summation mode; auto gain on, or the equivalent.
- Pipettes and pipette tips.
- 100 mL and 1 liter graduated cylinders.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution.
- Human IL-15 Controls (optional; available from R&D Systems).

PRECAUTION

Calibrator Diluent RD6-9 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

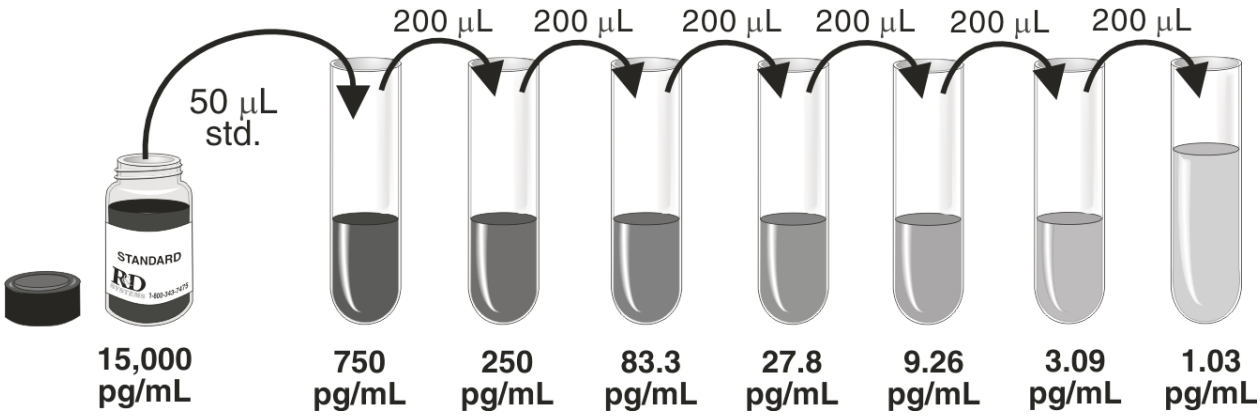
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 1000 mL of Wash Buffer.

Working Glo Reagent - Mix together 1 part Glo Reagent A (4 mL) and 2 parts Glo Reagent B (8 mL) 15 minutes to 4 hours before use in a capped plastic container and protect from light. 100 µL of the resultant mixture is required per well.

Note: *If running the assay in less than 96 wells, mix appropriate amounts of Glo Reagent A and Glo Reagent B. To assay half a plate (48 wells), mix 2 mL of Glo Reagent A with 4 mL of Glo Reagent B. Working Glo Reagent should be discarded after use.*

Standard - Reconstitute Standard with 1 mL of deionized or distilled water. This reconstitution produces a stock solution of 15,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 950 µL of Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-9 (*for serum/plasma samples*) into the 750 pg/mL tube. Pipette 400 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 750 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 50 µL of Assay Diluent RD1-19 to each well.

4. Add 100 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.

5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 200 µL of IL-15 Conjugate to each well. Cover with a new adhesive strip. Incubate for 3 hours at room temperature on the shaker.

   Note: *Prepare Working Glo Reagent at this time.*

7. Repeat the aspiration/wash as in step 5.

8. Add 100 µL of Working Glo Reagent to each well. Incubate for 5 - 20 minutes at room temperature **on the benchtop. Protect from light.**

9. Determine the RLU of each well using a luminometer set with the following parameters: 1.0 min. lag time; 0.5 sec/well read time; summation mode; auto gain on.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and Standards as instructed.

2. Add 50 µL Assay Diluent to each well.

3. Add 100 µL Standard, control, or sample to each well. Incubate for 2 hours on the shaker at RT.

4. Aspirate and wash each well 4 times.

5. Add 200 µL Conjugate to each well. Incubate for 3 hours on the shaker at RT.

Note: *Prepare Working Glo Reagent at this time.*

6. Aspirate and wash each well 4 times.

7. Add 100 µL Working Glo Reagent to each well. Incubate for 5 - 20 minutes **on the benchtop. Protect from light.**

8. Determine the RLU of each well using a luminometer.

CALCULATION OF RESULTS

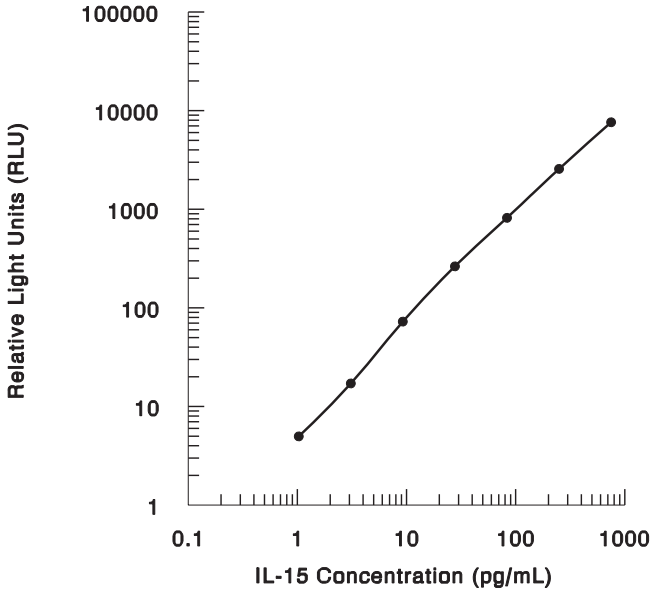
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard RLU.

Create a standard curve by reducing the data using computer software capable of generating a cubic-spline curve fit. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

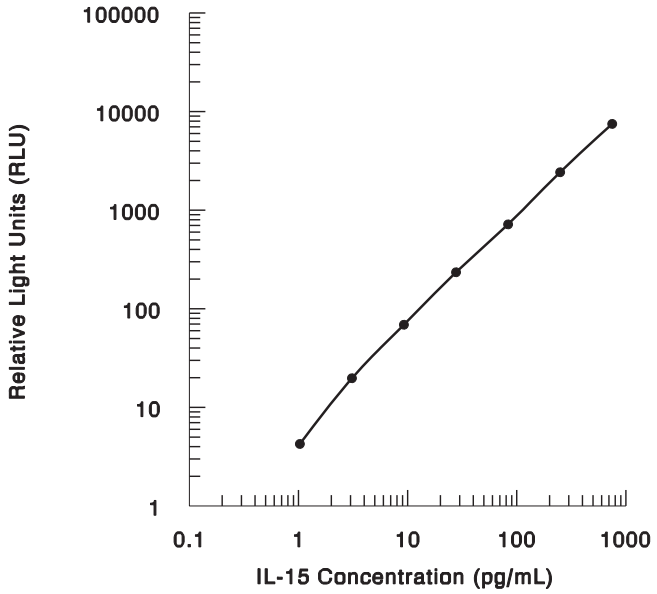
These standard curves were generated using a DYNEX TECHNOLOGIES MLX luminometer and are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD5R



pg/mL	RLU	Average	Corrected
0	0.520 0.532	0.526	—
1.03	5.010 5.988	5.499	4.973
3.09	17.63 17.65	17.64	17.11
9.26	72.45 73.78	73.11	72.58
27.8	262.5 266.0	264.2	263.7
83.3	814.2 822.8	818.5	818.0
250	2565 2567	2566	2565
750	7529 7722	7625	7625

Calibrator Diluent RD6-9



pg/mL	RLU	Average	Corrected
0	0.758 0.767	0.763	—
1.03	4.534 5.503	5.019	4.256
3.09	19.01 22.04	20.52	19.76
9.26	67.99 71.45	69.72	68.96
27.8	228.6 242.4	235.5	234.7
83.3	678.3 760.5	719.4	718.6
250	2390 2469	2430	2429
750	7367 7631	7499	7498

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Serum/Plasma Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	4.89	73.9	499	4.93	61.3	456
Standard deviation	0.26	2.90	18.0	0.65	7.69	41.1
CV (%)	5.2	3.9	3.6	13.3	12.6	9.0

Cell Culture Supernate Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	5.90	60.3	420	4.59	55.4	419
Standard deviation	0.22	1.80	11.7	0.60	5.82	27.4
CV (%)	3.7	3.1	2.8	13.1	10.5	6.5

RECOVERY

The recovery of IL-15 spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	97 - 110%
Serum (n=4)	105	90 - 119%
EDTA plasma (n=4)	105	87 - 114%
Heparin plasma (n=4)	105	88 - 119%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of IL-15 in various matrices were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	96	101	101	101
	Range (%)	93 - 101	89 - 113	89 - 110	93 - 112
1:4	Average % of Expected	95	96	99	98
	Range (%)	93 - 100	90 - 101	89 - 112	94 - 103
1:8	Average % of Expected	93	97	99	94
	Range (%)	92 - 97	92 - 101	93 - 105	91 - 97
1:16	Average % of Expected	94	97	96	95
	Range (%)	91 - 97	93 - 102	93 - 99	91 - 103
1:32	Average % of Expected	93	90	91	89
	Range (%)	86 - 98	84 - 98	89 - 96	84 - 95

SENSITIVITY

Ninety-eight assays were evaluated. The minimum detectable dose (MDD) of IL-15 ranged from 0.026 to 0.431 pg/mL. The mean MDD was 0.107 pg/mL.

The MDD was determined by adding two standard deviations to the mean RLU of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-15 produced at R&D Systems.

The NIBSC/1st WHO Reference Reagent recombinant human IL-15 preparation 95/554 was evaluated in this kit. The dose response curve of this standard parallels the QuantiGlo standard curve. To convert sample values obtained with the QuantiGlo Human IL-15 kit to approximate NIBSC 95/554 International units, use the equation below.

NIBSC (95/554) approximate value (IU/mL) = 0.0118 x QuantiGlo Human IL-15 value (pg/mL).

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of IL-15 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Std. Dev. (pg/mL)
Serum (n=39)	1.71	1.05 - 3.77	0.59
EDTA plasma (n=39)	1.64	0.98 - 3.23	0.53
Heparin plasma (n=39)	1.74	1.01 - 3.36	0.46

Cell Culture Supernates -
Human peripheral blood leukocytes (PBLs; 1 x 10^6 cells/mL) were cultured in RPMI and supplemented with 10% fetal bovine serum. The cells were cultured unstimulated or stimulated with 10 μg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 6 and assayed for levels of human IL-15.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	ND	ND
Stimulated	ND	1.09

ND = Non-detectable

Human cutaneous T cells (HuT-102) were cultured in RPMI and supplemented with 10% fetal bovine serum. An aliquot was removed, assayed for levels of human IL-15, and measured 14.6 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human IL-15. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range rhIL-15 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	**Recombinant mouse:**	**Other recombinants:**
IL-2	IL-2	bovine IL-2
IL-2 sRα	IL-15 Rα	canine IL-2
IL-2 sRβ		cotton rat IL-2
IL-2 sRγ		equine IL-2
		feline IL-2
		porcine IL-2
		rat IL-2

Recombinant mouse IL-15 cross-reacts 5.2% at 50 ng/mL.

Recombinant human IL-15 R/Fc Chimera interferes at levels $\geq$ 50 ng/mL.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.