

Human IL-15 ELISA Kit

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

Catalogue Number: EL10044

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES



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INTENDED USE

This Human Interleukin-15 ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin 15 (IL-15) concentrations in serum, plasma, and cell culture supernatant. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Interleukin-15 (IL-15), a modest 14-15 kDa glycoprotein, is produced by monocytes and dendritic cells and at maturity reaches 114 amino acid residues in length. IL-15 is a member of the four α helix bound cytokine family that includes IL-2, IL-3, IL-5, IL-6, IL-7 and IL-9. Although the structure of IL-15 has not been determined, it is predicted to be similar to IL-2 and other members of the four-helix bundle cytokine family. IL-15 mRNA has been discovered in the placenta, skeletal muscle, kidneys, lungs, heart, fibroblasts, epithelial cells, dendritic cells, and monocytes. Human IL-15 shares approximately 97% and 73% amino acid sequence identity with simian and mouse IL-15, respectively. Both human and simian IL-15 molecules are active with mouse cells.

The biological activities of IL-15 are comparable to IL-2: IL-15 induces the proliferation of activated CD4-CD8-, CD4+CD8+, CD4+, and CD8+ cells and dendritic epidermal T cells. IL-15 co-stimulates proliferation of B cells activated with immobilized anti-human IgM or phorbol ester, but has no stimulatory effect on resting B cells. In combination with recombinant CD40L, IL-15 is a potent inducer of polyclonal IgM, IgG1, and IgA secretion, but does not cause production of IgG4 or IgE. The development, survival, and activation of natural killer (NK) cells is related to IL-15. IL-15 also affects the non-lymphoid tissues, such as muscle, brain, microglia, and mast cells. Abnormal levels of IL-15 have been reported in rheumatoid arthritis, inflammatory bowel disease, and in diseases associated with the retroviruses HIV and HTLV-I.

This IL-15 ELISA is a ready-to-use 3.5-hour solid phase immunoassay readily capable of measuring IL-15 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 2000 pg/mL. This assay has shown no cross-reactivity with other cytokines tested, and is expected to be used effectively for further investigations into the relationship between IL-15 and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This IL-15 enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to IL-15. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-15 and incubated. IL-15 if present, will bind and become immobilized by the antibody pre-coated on the wells and then be “sandwiched” by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound IL-15 and other components of the sample. In order to quantitatively determine the amount of IL-15 present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin and a TMB (3,3',5,5' tetramethylbenzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain IL-15, biotin-conjugated antibody, and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of IL-15 in the samples, this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus IL-15 concentration (pg/mL). The concentration of IL-15 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF APPLICATION

- The Human IL-15 ELISA kit is not for use in clinical diagnostic procedures, and for laboratory use only.
- Although all manufacturing precautions have been exercised to ensure that this product will be suitable for use with all validated sample types as designated in the product insert, the possibility of interference cannot be excluded due to the variety of proteins that may exist within the sample.
- The Calibrator Diluent selected for the standard curve should be consistent with the assay samples. If the values generated by the samples are greater than the uppermost standard, the samples dilution should be adjusted with the appropriate Calibrator Diluent and the assay should be repeated.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests
1.	IL-15 MICROTITER PLATE (Part EL44-1) _____ 96 wells Pre-coated with anti-human IL-15 monoclonal antibody.
2.	BIOTIN CONJUGATE (Part EL44-2) _____ 6 mL Anti-human IL-15 polyclonal antibody conjugated to Biotin.
3.	AVIDIN CONJUGATE (Part EL44-3) _____ 12 mL Avidin conjugated to horseradish peroxidase.
4.	IL-15 STANDARD (Part EL44-4) _____ 2 vials Recombinant human IL-15 (4000 pg/vial) in a buffered protein base with preservative, lyophilized.
5.	CALIBRATOR DILUENT I (EL44-5) _____ 25 mL Animal serum with buffer and preservative. <i>For serum/plasma testing.</i>
6.	CALIBRATOR DILUENT II (EL44-6) _____ 25 mL Cell culture medium with calf serum and preservative. <i>For cell culture supernatant testing.</i>
7.	WASH BUFFER (20X) (Part 30005) _____ 60 mL 20-fold concentrated solution of buffered surfactant.
8.	SUBSTRATE A (EL44-7) _____ 10 mL Buffered solution with H ₂ O ₂
9.	SUBSTRATE B (Part 30007) _____ 10 mL Buffered solution with TMB.
10.	STOP SOLUTION (Part 30008) _____ 14 mL 2N Sulphuric Acid (H ₂ SO ₄). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μ L and 50-200 μ L for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm \pm 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
Solid Wastes: autoclave for 60 minutes at 121°C.
Liquid Wastes: add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat and flame.

12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

- a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
- b) **Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
- c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulate. *This IL-15 ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, EDTA, or heparin.*

Note: Citrate plasma is not recommended for use in this EIA assay.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, and cell culture supernatant samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

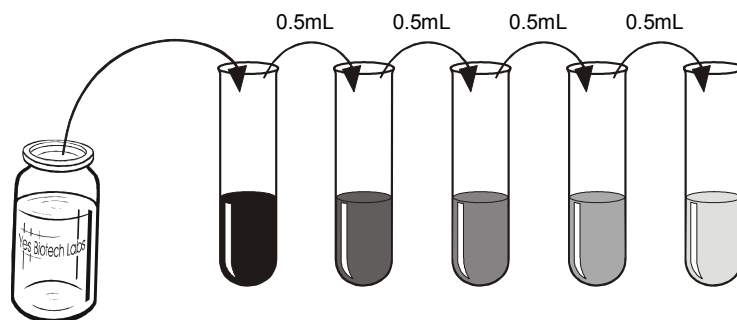
1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes within 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-15 Standard:**

- a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute IL-15 Standard with 2.0 mL of Calibrator Diluent I (for serum / plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 2000 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IL-15 standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.
- b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (0 to 2000 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-15 stock solution will serve as the high standard (2000 pg/mL) and the Calibrator Diluent will serve as the zero-standard (0 pg/mL).



IL-15 Standard	1,000pg/mL	500pg/mL	250pg/mL	125pg/mL	62.5pg/mL	2,000pg/mL
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ASSAY PROCEDURE

1. Prepare Wash Buffer and IL-15 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
1A, 1B	Standard 1 - 0 pg/mL (S1)	2A, 2B	Standard 5 - 500 pg/mL (S6)
1C, 1D	Standard 2 - 62.5 pg/mL (S2)	2C, 2D	Standard 6 - 1000 pg/mL (S7)
1E, 1F	Standard 3 - 125 pg/mL (S3)	2E, 2F	Standard 7 - 2000 pg/mL (S7)
1G, 1H	Standard 4 - 250 pg/mL (S4)	3A-12H	IL-15 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 50 μ L of Anti-IL15 biotin conjugate to the antibody pre-coated Microtiter Plate
3. Add 100 μ L of Standard or Sample to the appropriate wells. Mix well. Cover and Incubate for 2 hours at room temperature.
4. Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μ L/well/wash (range: 350-400 μ L). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.
5. Add 100 μ L Avidin Conjugate to each well. Cover and incubate for 1 hours at room temperature.

6. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).
7. Repeat wash procedure as described in Step 4.
8. Add 100 μ L Substrate Solution into each well. Cover and Incubate for 15 minutes at room temperature.
9. Add 100 μ L Stop Solution to each well. Mix well.
10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-15 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-15 concentration (pg/mL) on the horizontal (X) axis.

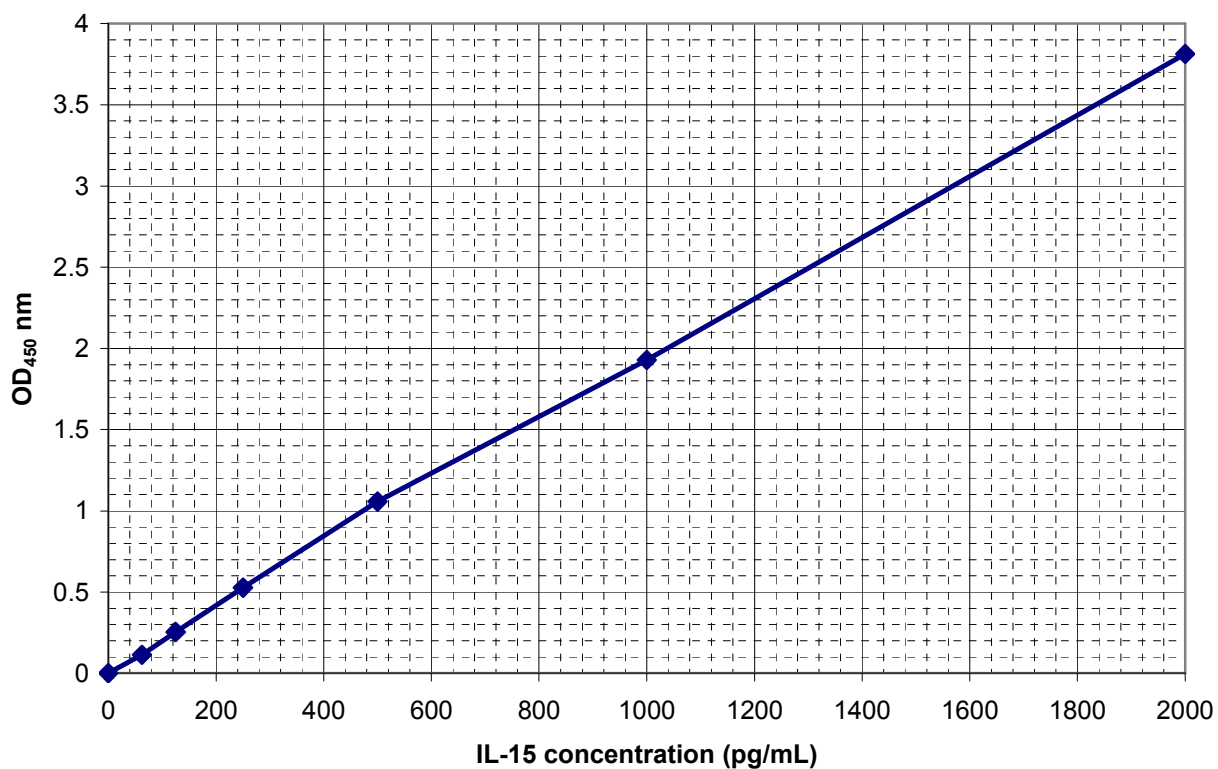
1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of IL-15 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-15 concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

Results of a typical standard run of a IL-15 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate their own results.

EXAMPLE

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.008, 0.074	0.041	0
62.5	0.155, 0.155	0.155	0.114
125	0.299, 0.289	0.294	0.253
250	0.559, 0.577	0.568	0.527
500	1.084, 1.091	1.098	1.057
1000	1.944, 1.994	1.969	1.928
2000	3.841, 3.869	3.855	3.814



PERFORMANCE CHARACTERISTICS

1. RECOVERY

By employing various samples, the recovery of IL-15 was evaluated with 7 different amounts of IL-15 throughout the range of the assay. All samples were mixed and assayed in duplicate.

Sample Type	Average Recovery (%)	Range (%)
Cell Culture Media	98	84-110
Serum	99	92-109
EDTA plasma	96	93-102
Heparin plasma	102	94-108

2. SENSITIVITY

The minimum detectable quantities of human IL-15 are 10 pg/mL and 9 pg/mL, when use a standard curve generated with Calibrator Diluent I and Calibrator Diluent II, respectively. The two standard deviations above the mean optical density of the 20 replicates of the zero standard were defined as the minimum detectable quantities

3. SPECIFICITY

This sandwich ELISA can detect both natural and recombinant human IL-15. This kit exhibits no significant cross-reactivity with human IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, TNF- α , TNF- β , TGF- β 1, TGF- β 2, G-CSF, GM-CSF.

4. CALIBRATION

This immunoassay is calibrated against recombinant human IL-15. (NIBSC/WHO First International Standard 95/554).

REFERENCES

1. Agostini, C. et al. (1996) *J. Immunol.* 157, 910–918
2. Armitage, R.J. et al. (1995) *J. Immunol.* 154, 483–490
3. Azimi, N. et al. (1999) *J. Immunol.* 163, 4064–4072.
4. Bazan, J.F. (1990) *Proc. Natl Acad. Sci. USA* 87, 6934–6938
5. Bamford, R.N. et al. (1996a) *Proc. Natl Acad. Sci. USA* 93, 2897–2902
6. Burton, J.D. et al. (1994) *Proc. Natl Acad. Sci. USA* 91, 4935–4939
7. Carson, W.E. et al. (1994) *J. Exp. Med.* 180, 1395–1403
8. Edelbaum, D. et al. (1995) *J. Invest. Dermatol.* 105, 837–843
9. Garcia, V.E. et al. (1998) *J. Immunol.* 160, 4322–4329
10. Grabstein, K.H. et al. (1994) *Science* 264, 965–968
11. Hanisch, U.K. et al. (1997) *J. Biol. Chem.* 272, 28853–28860
12. Kacani, L. et al. (1997) *Clin. Exp. Immunol.* 108, 14–18
13. Kakumu, S. et al. (1997) *Clin. Exp. Immunol.* 109, 458–463
14. Kivisakk, P. et al. (1998) *Clin. Exp. Immunol.* 111, 193–197
15. Lee, Y.B. (1996) et al. (1996) *Neuroreport* 7, 1062–1066
16. McInnes, I.B. et al. (1996) *Nature Med.* 2, 175–182
17. Mingari, M.C. et al. (1997) *Eur. J. Immunol.* 27, 1374–1380
18. Mrózek, E. et al. (1996) *Blood* 87, 2632–2640
19. Ogasawara, K. et al. (1998) *Nature* 391, 700–703
20. Quinn, L.S. et al. (1997) *Biochem. Biophys. Res. Commun.* 239, 6–10
21. Suzuki, H. et al. (1997) *J. Exp. Med.* 185, 499–505
22. Tagaya, Y. et al. (1996a) *Immunity* 4, 329–336
23. Tagaya, Y. et al. (1996b) *EMBO J.* 15, 4928–4939
24. Zhang, X. et al. (1998) *Immunity* 8, 591–599