

Human IL-15 ELISA Kit

For the quantitative determination of human Interleukin-15 (IL-15) concentrations in serum, plasma, and 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) is a novel cytokine that shares many biological properties with but lacks amino acid sequence homology to, IL-2. IL-15 was originally identified in media conditioned by a monkey kidney epithelial cell line (CV1/EBNA) based on its mitogenic activity on the murine T cell line, CTLL-2. IL-15 was also 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.

Human, simian and mouse IL-15 cDNA, as well as human and mouse IL-15 genomic clones, have been isolated and characterized. The IL-15 cDNA clones from all three species encode a 162 amino acid (aa) residue precursor protein containing a 48 aa residue leader that is cleaved to generate the 114 aa residue mature IL-15. Human IL-15 shares approximately 97% and 73% sequence identity with simian and mouse IL-15, respectively. Both human and simian IL-15 are active on mouse cells. 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 has biological activities similar to IL-2 and has been shown to stimulate the growth of natural killer cells, activated peripheral blood T lymphocytes tumor infiltrating lymphocytes (TILs) and B cells. In addition, IL-15 has also been shown to be a chemoattractant for human blood T lymphocytes and to be able to induce lymphokine-activated killer (LAK) activity in NK cells as well as to be able to induce the generation of cytolytic effector cells. It is likely that additional, as yet unidentified, functions for IL-15 will be discovered in the future.

This IL-15 ELISA is a 4.5 hour solid phase immunoassay readily applicable to measure IL-15 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 1000 pg/mL. It showed no cross reactivity with other cytokines tested. This IL-15 ELISA is expected to be effectively used 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 $450 \text{ nm} \pm 2 \text{ nm}$. 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.

LIMITATIONA OF THE PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- The kit should not be used beyond the expiration date on the kit label.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of legends in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

	96 tests
1. IL-15 MICROTITER PLATE	96 wells
Pre-coated with anti-human IL-15 monoclonal antibody.	
2. BIOTIN CONJUGATE	7 mL
Anti-human IL-15 polyclonal antibody conjugated to Biotin.	
3. AVIDIN CONJUGATE	14 mL
Avidin conjugated to horseradish peroxidase.	
4. IL-15 STANDARD	2 vials
Recombinant human IL-15 (2000 pg/vial) in a buffered protein base with preservative, lyophilized.	
5. CALIBRATOR DILUENT I (Part 30003)	22 mL
Animal serum with buffer and preservative. <i>For serum/plasma testing.</i>	
6. CALIBRATOR DILUENT II (Part 30004)	22 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 (Part 30006)	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

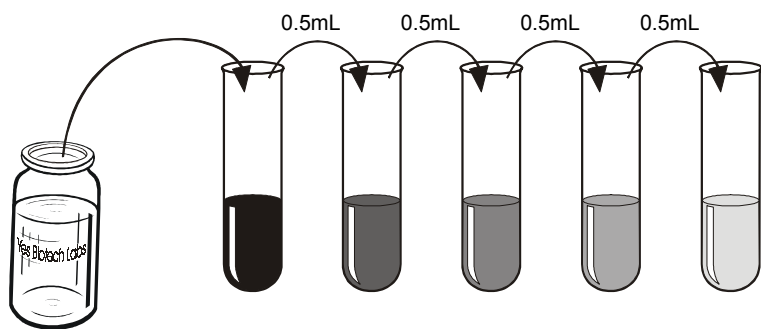
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

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 or Calibrator Diluent II. This reconstitution produces a stock solution of 1000 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 1000 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 (1000 pg/mL) and the Calibrator Diluent will serve as the zero-standard (0 pg/mL).



IL-15 Standard	500pg/ml	250pg/ml	125pg/ml	62pg/ml	31pg/ml
1,000pg/ml					

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 - 250 pg/mL (S6)
1C, 1D	Standard 2- 31 pg/mL	(S2)	2C, 2D Standard 6 - 500 pg/mL (S7)
1E, 1F	Standard 3- 62 pg/mL	(S3)	2E, 2F Standard 7- 1000 pg/mL (S7)
1G, 1H	Standard 4- 125 pg/mL	(S4)	3A-12H IL-10 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 μL of Anti-IL15 biotin conjugate to the antibody pre-coated Microtiter Plate
3. Add 100 μL 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 μL /well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.
5. Add 100 μL Avidin Conjugate to each well. Cover and incubate for 2 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 20 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 RESULT

The standard curve is used to determine the amount of MCAF 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 MCAF concentration (pg/mL) on the horizontal (X) axis.

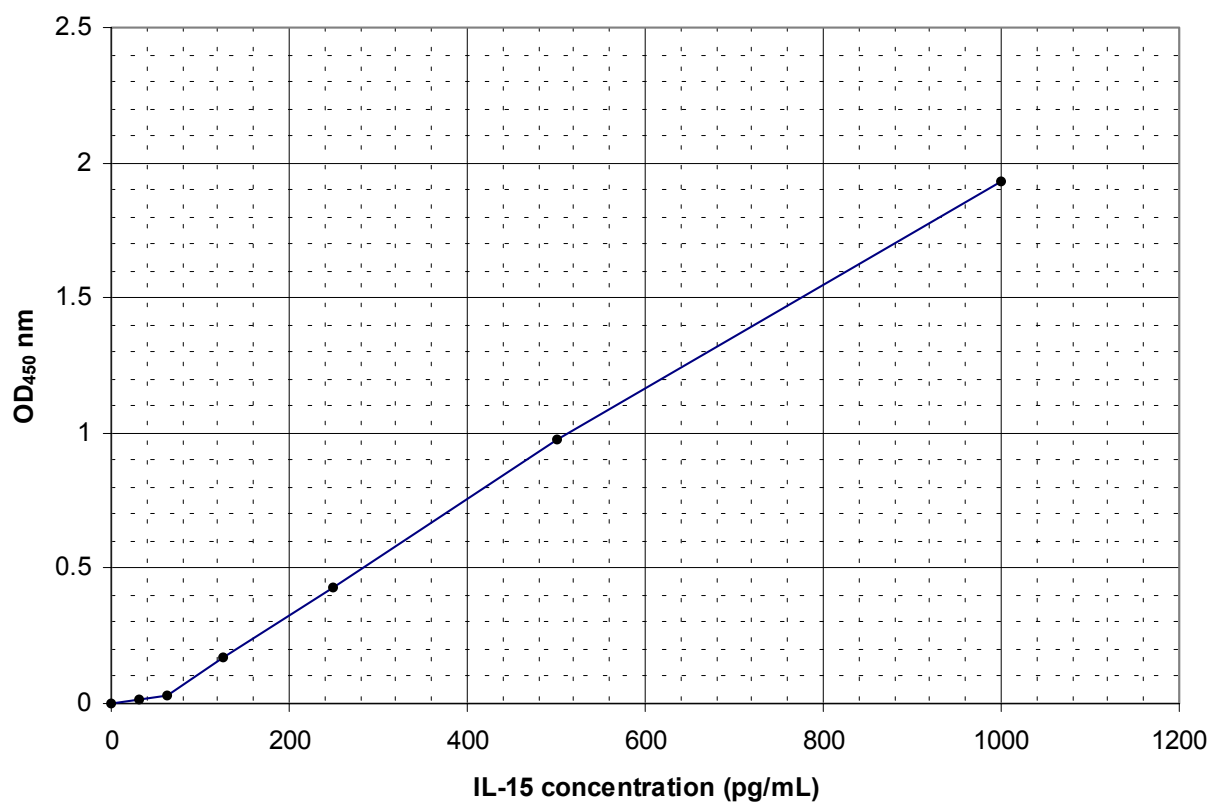
1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of MCAF in each sample, first 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 MCAF 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 MCAF 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	0.073, 0.067	0.070	0
31	0.092, 0.080	0.086	0.016
62	0.242, 0.232	0.098	0.028
125	0.499, 0.503	0.237	0.167
250	0.610, 0.595	0.501	0.431
500	1.033, 1.065	1.049	0.979
1000	1.996, 2.012	2.004	1.934



PERFORMANCE CHARACTERISTICS

1. RECOVERY

The recovery of IL-15 spiked to seven different levels in four test samples throughout the range of the assay was evaluated. All samples were mixed and assayed in duplicate.

<i>Sample Type</i>	<i>Average Recovery (%)</i>	<i>Range (%)</i>
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 dose of IL-15 was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human IL-15 using a standard curve generated with Calibrator Diluent I is <10 pg/mL and using Calibrator Diluent II is <9 pg/mL.

3. SPECIFICITY

This sandwich ELISA recognises 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 natural human IL-15. (NIBSC/WHO First International Standard 95/554).