Human IL-18 ELISA Kit

For the quantitative determination of human Interleukin-18
(IL-18) concentrations in biological samples.

Catalogue Number: EL10060
96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTENDED USE

This human IL-18 ELISA kit is to be used for the *in vitro* quantitative determination of human interleukin-18 (IL-18) concentrations in cell culture supernatant and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY.

INTRODUCTION

IL-18 was first identified and cloned as an interferon-γ (IFN-γ) inducing factor from a murine liver cell cDNA library after challenging the animal with heat inactivated bacteria and lipopolysaccharide[8]. It is categorized as pro-inflammatory cytokine based on its structural similarity to the interleukin 1 superfamily and its function in modulating inflammatory immune-response. IL-18 is constitutively expressed as a precursor in nearly all cells in human and animals. Sequencing human IL-18 revealed 65% homology with murine IL-18 and an unusual 35 amino acid leader peptide at its N-terminus. Similar to IL-1β, IL-18 precursor is processed by caspase-1 into the active form. The primary source of IL-18 is macrophages and dendritic cells and its secretion is increased by IFN-γ stimulation. Circulating IL-18 presents in healthy individuals at substantial levels.

IL-18 can act with IL-12 synergistically to stimulate T cells and natural killer cells to produce IFN-γ and augment T-helper 1 type immune-response[11]. It is also be shown that IL-12 and IL-18 induces anti-CD40-activated B cells to produce IFN-γ, inhibits IL-4 dependent IgE and IgG1 production and enhances IgG2a production without inhibiting the B cell proliferative response[12].

IL-18 acts through binding to its receptor IL-18Rα. Similar to that of IL-1β receptor complex, an accessory protein, known as IL-18 receptor β, can increase the responsiveness of IL-18Rα to IL-18 significantly. Experiments have shown that IL-12 and IL-1β can increase the expression of IL-18 receptor, and IL-18 receptor is selectively expressed on T-helper 1 cells, but not on T-helper 2 cells. Resembling that of IL-1, the IL-18 signal transduction involved with the sequential recruitment of MyD88, the four IRAKs and TRAF-6, followed by the degradation of IκB and release of NFκB[2]. The IL-18 activation of the receptor requires high ligand concentration at 10–20 ng/mL and sometime higher levels, which is a unique feature of this cytokine[2].

The function of IL-18 can be neutralized by a constitutively expressed and secreted IL-18 binding protein[1] (IL-18BP). IL-18BP has an immunoglobulin-like structure similar to soluble IL-1 receptor. However, IL-18BP does not have a transmembrane domain. Through binding to IL-18, IL-18BP suppresses the production of IFN-γ, resulting in reduced T-helper type 1 immune responses.

It has been reported that up-regulated IL-18 expression was implicated with autoimmune diseases (Crohn's disease[7], Hashimoto's thyroiditis[6], Eczema[5] etc.) and inflammatory conditions (increased risk of atherosclerotic plaque rupture and cardiovascular event in chronic kidney patients[4], Alzheimer's disease[9], age-related macular degeneration[3]). IL-18 may play a role in cancer's immune-evasion through inducing PD-1 expression in mature nature killer cells[10].

The IL-18 ELISA kit is for measurement of IL-18 in cell culture and other biological samples. The kit is for research only. The detected value of IL-18 can only be used as reference for scientific study. The kit should not be used in any diagnostic or therapeutic procedures.

PRINCIPLE OF THE ASSAY

This 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 for human IL-18. When standards or samples are added to the appropriate microtiter plate wells, human IL-18 in the standards or samples will be immobilized by the pre-coated antibody during incubation. A biotin-conjugated antibody preparation specific for human IL-18 is added to each well and incubated. The biotin labelled antibody attaches to the wells by binding to human IL-18. After plate washing, other proteins, components and unattached biotin labelled antibody is removed. Then, avidin-horseradish peroxidase (HRP) conjugate is added to each well. Avidin has a very high affinity for biotin, thus, it links the tracer (HRP) sturdily to the biotin labelled antibody. The wells are thoroughly washed to remove all unbound avidin-HRP conjugate and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only wells that contain human IL-18 will exhibit a change in colour. The extent of colour change is proportional to the quantity of human IL-18 presented in the standards/samples. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wave length of 450 nm ± 2 nm.

According to the testing system, the provided standard is diluted (2-fold) with the appropriate diluent and assayed at the same time as the samples. This allows the operator to produce a curve of Optical Density (O.D.) versus human IL-18 concentration (pg/mL) in standards. The concentration of human IL-18 in the samples is then determined by comparing the O.D. of the samples to the standard curve and multiplying with sample dilution factor.

REAGENTS PROVIDED

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

96 tests

1. **HUMAN IL-18 MICROTITER PLATE** (Part EL60-1)________________ ***96 wells***
   Pre-coated with anti-human IL-18 monoclonal antibody

2. **BIOTIN CONJUGATE** (Part EL60-2) ________________________________ ***6 mL***
   Anti-human IL-18 monoclonal antibody conjugated to biotin

3. **AVIDIN-HRP CONJUGATE** (Part EL60-3)__________________________ ***12 mL***
   Avidin conjugated to horseradish peroxidase

4. **HUMAN IL-18 STANDARD** (Part EL60-4)__________________________ ***2 vials***
   Recombinant human IL-18 (4000pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL60-5)___________________________ ***25 mL***
   Buffered animal serum with preservative

6. **CALIBRATOR DILUENT II** (Part EL60-6____________________________ ***25 mL***
   Cell culture medium with bovine serum and preservative

7. **WASH BUFFER (20X)** (Part 30005)_________________________________ ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **TMB SUBSTRATE** (Part 30010)____________________________________ ***11 mL***
   TMB solution

9. **STOP SOLUTION** (Part 30008)_____________________________________ ***14 mL***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips (10-100μL and 50-200μL) for running the assay.
2. Pipettes: 1 mL, 5 mL, and 10 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Eppendorf tubes and racks.
5. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
6. Microtiter plate reader (450 nm ± 2nm)
7. Automatic microtiter plate washer or squirt bottle.
8. Sodium hypochlorite solution, 5.25% (household liquid bleach).
9. Deionized or distilled water.
10. Plastic plate cover.
11. Disposable gloves.
12. 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. All biological samples should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn and good laboratory practices should be followed during the assay procedure.
9. All biological samples should be disposed of in a manner that will inactivate 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. 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

STORAGE AND DILUTION:

Samples should be assayed immediately, or be stored at -20°C or lower temperature for long term storage. Bring samples to room temperature prior to the assay. Use CALIBRATOR DILEUNT or a buffer similar to the samples to dilute standards and samples.

PREPARATION OF REAGENTS

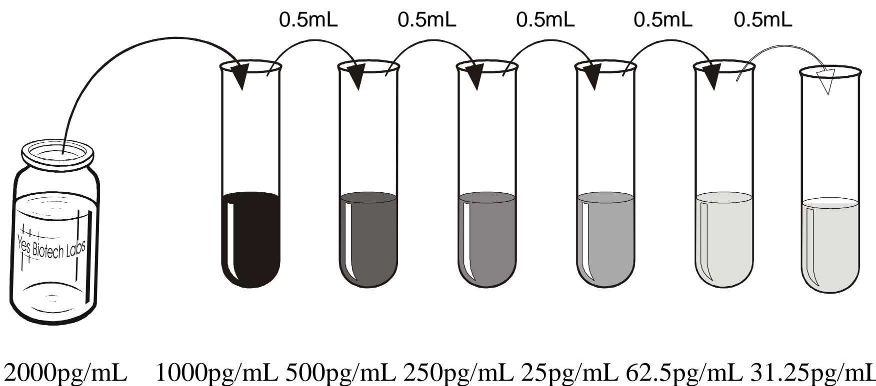
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as below.

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.

2. **Human IL-18 Standard:** Two vials of IL-18 standard are included in the kit. The standard is a human cell expressed recombinant IL-18 protein.

   a) Reconstitute the **Human IL-18 Standard** with 2mL of appropriate Calibrator Diluent. This reconstitution produces a stock solution of 2000pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstitution. Aliquot the stock and store frozen at -20°C (short term) or -70°C (long term) if repeated use is expected.

   b) Use the above stock solution to produce a 2-fold serial dilution within the range of this assay (31.25pg/mL to 2000pg/mL) as illustrated. Between each test tube transfer, be sure to mix contents thoroughly. The undiluted human IL-18 standard stock will serve as the high standard (2000pg/mL) and the Calibrator Diluent will serve as the zero-standard (0pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X), human IL-18 Standards and Samples before starting assay procedure (Refer to Sample Preparation and Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0pg/mL** (S1)	**2A, 2B**	Standard 5 – **250pg/mL** (S5)
1C, 1D	Standard 2 – **31.25pg/mL** (S2)	**2C, 2D**	Standard 6 – **500pg/mL** (S6)
1E, 1F	Standard 3 – **62.5pg/mL** (S3)	**2E, 2F**	Standard 7 – **1000pg/mL** (S7)
1G, 1H	Standard 4 – **125pg/mL** (S4)	**2G,2H**	Standard 8 – **2000pg/mL** (S8)
3A….12H			

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 50μL of Standards or Samples to the appropriate wells of anti-human IL-18 antibody pre-coated microtiter plate. Add 50μL of anti-human IL-18 Biotin Conjugate to each well. Mix briefly on ELISA plate shaker.

3. Cover and incubate plate for 2 hour 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 350μL/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. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100µL of Avidin-HRP conjugate to each well. Cover and incubate for 1 hour at room temperature.

6. Repeat wash procedure as described in Step 4.

7. Add 100µL TMB Substrate to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100µL Stop Solution to each well. Mix well.

9. 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 human IL-18 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 human IL-18 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 (0pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of human IL-18 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 human IL-18 concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

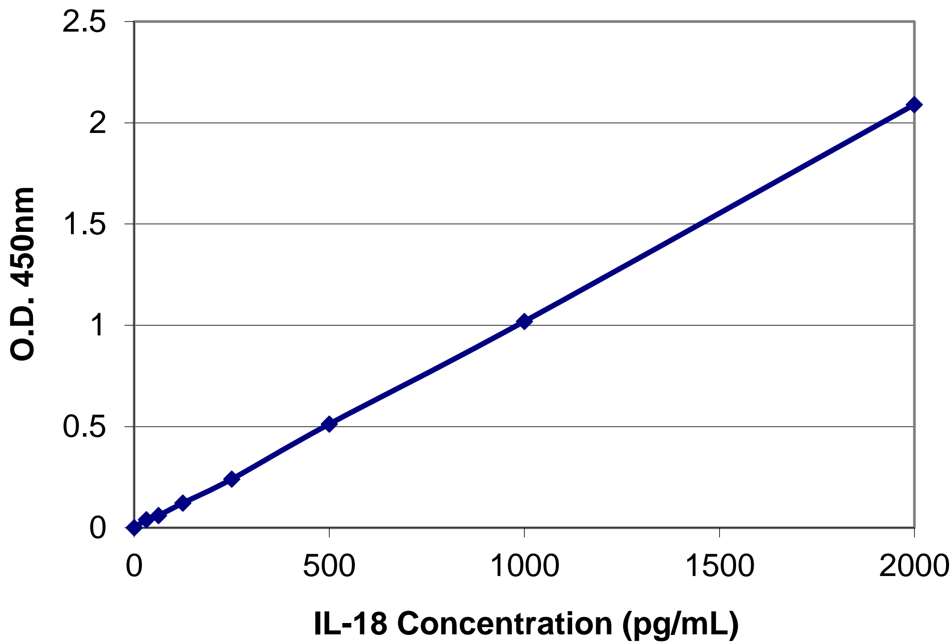
TYPICAL DATA

Results of a typical standard run of a human IL-18 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 user results.

EXAMPLE I

The following data and standard curve were obtained using CALIBRATOR DILUENT I.

Standard (pg/mL)	Mean O.D. (450 nm)	Zero Standard Subtracted (Std.)-(S1)
0	0.069	0.000
31.25	0.109	0.040
62.5	0.130	0.061
125	0.191	0.122
250	0.309	0.240
500	0.581	0.512
1000	1.088	1.019
2000	2.159	2.090

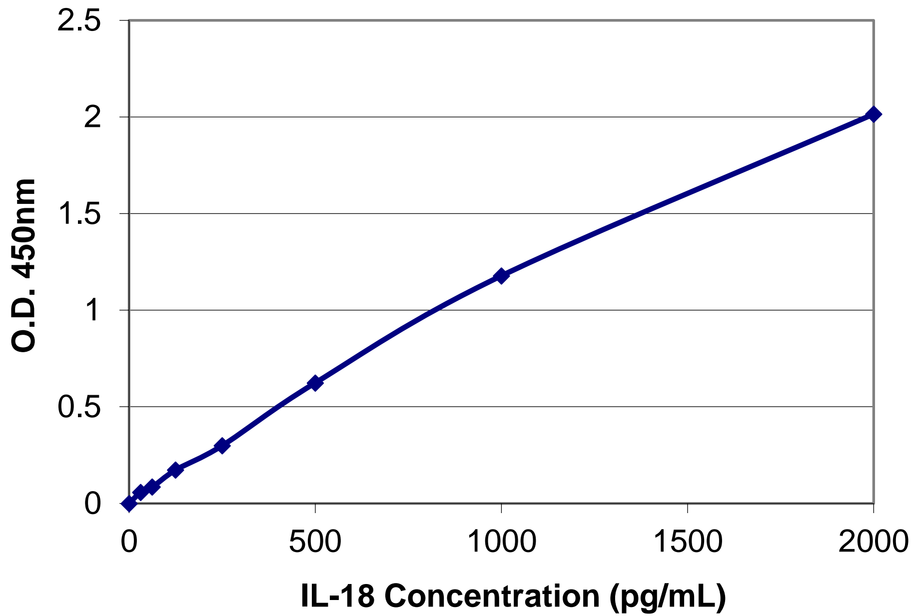


EXAMPLE II

The following data and standard curve were obtained using CALIBRATOR DILUENT II.

Standard (pg/mL)	Mean O.D. (450 nm)	Zero Standard Subtracted (Std.)-(S1)
0	0.088	0.000
31.25	0.146	0.058
62.5	0.173	0.085

125	0.261	0.173
250	0.387	0.299
500	0.712	0.624
1000	1.266	1.178
2000	2.103	2.015



RFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of IL-18 at low, medium and high level, were assayed in 16 replicates in 1 assay.

Sample	1	2	3
N	16	16	16
Mean (pg/ml)	69.4	263.25	1008.3
Coefficient of Variation (%)	6.0	3.2	2.8

2. **INTER-ASSAY PRECISION**

To determine between-run precision, three different samples of IL-18 at low, medium and high level, were measured in 8 different assays.

Sample	**1**	**2**	**3**
N	8	8	8
Mean (pg/mL)	62	251	976.5
Coefficient of Variation (%)	7.6	4.3	2.8

3. **RECOVERY**

1). Recovery in human serum samples:

Human serum samples were spiked with different concentrations of recombinant IL-18. The list below is the recovery rates of IL-18 at low, medium and high level.

IL-18 added to serum	**Range of Recovery Rate%**	**Average of Recovery Rate %**
1000 pg/mL	101-102%	102%
250 pg/mL	88-90%	89%
62.5 pg/mL	93-104%	98.5%

2). Recovery in cell culture medium:

Cell culture medium was spiked with different concentrations of recombinant IL-18. The list below is the recovery rates of IL-18 at low, medium and high level.

IL-18 added to culture medium	**Range of Recovery Rate%**	**Average of Recovery Rate %**
1000 pg/mL	96-101%	98%
250 pg/mL	95-102%	98%
62.5 pg/mL	108-112%	110%

4. **SENSITIVITY**

The minimum detectable dose of human IL-18 in serum was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human IL-18 calculated from calibrate diluent I diluted standard curve was 3.25pg/mL.

5. **CROSS-REACTIVITY**

The kit was tested with 32 different native and recombinant human proteins. No cross-reactivity were found.

6. **SAMPLE TEST**

25 normal serum samples were tested with the kit. The OD of one sample was out of curve range. The average concentration of IL-18 in the rest 24 normal serum samples was 162pg/mL. 69 normal plasma samples were tested. The ODs of 3 plasma samples were out of curve range. The average concentration of IL-18 in the rest 66 plasma samples was 271pg/ML.

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