Immunoassay Kit
Catalog #KHC0181

*Human*
# IL-18

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## INTENDED USE

The Invitrogen Human Interleukin-18 (Hu IL-18) ELISA kit is to be used for the *in vitro* quantitative determination of Hu IL-18 in human serum, EDTA plasma, cell culture supernatant, and buffered solution. The assay will recognize both natural and recombinant Hu IL-18.

## INTRODUCTION

IL-18, also known as Interferon-gamma Inducing Factor (IGIF) is a cytokine with $M_r$=18 kDa (157 amino acid residues) produced by macrophages and monocytes, Kuppfer cells, keratinocytes, intestinal epithelial cells, osteoblasts, mouse diencephalon, and adrenal cortical cells of reserpine-treated rats. IL-18 is synthesized as an inactive precursor molecule with $M_r$=24 kDa which lacks a signal peptide. The IL-18 precursor is cleaved by IL-1 converting enzyme (ICE, Caspase-1), producing the bioactive, mature form. Only the mature, 18 kDa, form of IL-18 is secreted. Cells that respond to IL-18 include Th1-type cells and NK cells.

IL-18 exerts several effects on Th1-like cells. IL-18 stimulates Th1 cell proliferation, Fas ligand expression and IL-2R alpha chain expression. IL-18 also works in combination with IL-12 to induce the production of interferon-gamma, GM-CSF, and IL-2 by Th1-type cells.

Standard bioassays for IL-18 measure dose dependent interferon-gamma production by IL-18 target cells, such as KG-1 cells (human myelomonocyte: ATCC CCL246). Immunomodulatory pathways which include IL-18 stimulation of interferon-gamma production are under investigation. Interferon-gamma production by Th1-type cells and NK cells is important in many immune functions, including defense against viral and parasitic infections; enhancement of

NK activity; activation of macrophages; enhancement of B cell function including B cell maturation, proliferation and immunoglobulin secretion; enhancement of MHC class I and class II antigen expression; and inhibition of osteoclast activation.

IL-18 is implicated as a mediator of septic shock and tissue injury in response to inflammation, and is implicated in some forms of diabetes. Septic shock induced by challenge with lipopolysaccharides and liver damage found in mice following treatment with *Propionibacterium acnes* can be abrogated by administration of anti-IL-18 antibodies. IL-18 is overexpressed in autoimmune non-obese diabetic mice (NOD).

**This kit has been configured for research use only and is not to be used in diagnostic procedures.**

## Read entire protocol before use.

### PRINCIPLE OF THE METHOD

The Invitrogen Hu IL-18 ELISA kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu IL-18 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu IL-18 content, control specimens, and unknowns, are pipetted into these wells, and incubated for one hour. During this first incubation, Hu IL-18 is captured and immobilized onto the plate. After washing, a horseradish peroxidase (HRP) conjugated anti-Hu IL-18 antibody is pipetted into the wells, and incubated for one hour. The binding of the HRP conjugated antibody to the wells completes a three-member solid phase sandwich. After a second washing, the HRP activity is detected by addition of TMB Substrate Reagent. Incubation of the Substrate

Reagent within the wells produces color which is proportional to the quantity of Hu IL-18 contained in the sample. At the end of the incubation period, Stop Solution is added to each well which terminates the HRP catalyzed reaction and stabilizes the color formed. The absorbance of each well is measured at 450 nm using a microtiter plate reader.

**REAGENTS PROVIDED**

**Note**: *Store all reagents at 2 to 8°C.*

| ***Reagent*** | ***96 Test Kit*** |
|---|---|
| Hu IL-18 Calibrator, lyophilized. Refer to Lot Specific Package Insert for mass and reconstitution volume. Contains 0.1% sodium azide. | 2 vials |
| Assay Diluent. Contains 0.1% sodium azide; 30 mL per bottle. | 1 bottle |
| Hu IL-18 Antibody-Coated Wells, 96 wells per plate. | 1 plate |
| Hu IL-18 Conjugate Reagent, (101x concentrate); 0.2 mL per vial. | 1 vial |
| Conjugate Diluent, 24 mL per bottle. | 1 bottle |
| Wash Solution Concentrate (10x); 100 mL per bottle. | 1 bottle |
| Substrate Reagent (TMB/$H_2O_2$); 20 mL per bottle. | 1 bottle |
| Stop Solution (0.5 mol/L $H_2SO_4$) (irritant); 20 mL per bottle. | 1 bottle |

**Disposal Note:** This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

## SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips.
3. Calibrated multi-channel pipette.
4. Distilled or deionized water.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
7. Glass or plastic tubes for diluting and aliquoting standard.
8. Absorbent paper towels.
9. Calibrated beakers and graduated cylinders in various sizes.
10. 96-well polyvinyl plate.
11. Microplate holder.

## PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the zip-lock bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes. Fresh samples should be used when possible. If storage is necessary, samples should be apportioned into aliquots and stored at -20°C. Thaw samples completely and mix well prior to analysis. Avoid repeated freeze-thaw cycles. Samples may be diluted in Assay Diluent. For example, sera may be diluted 1:5 by adding 50 µL of sample to 200 µL Assay Diluent. Multiply results obtained by the appropriate dilution factor.
4. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
5. It is recommended that all standards, controls and samples be run in duplicate.
6. Samples that are >1000 pg/mL should be diluted with Assay Diluent and reanalyzed.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. **Do not mix or interchange different reagent lots from various kit lots**.
10. Do not use reagents after the kit expiration date.

11. Read absorbances within 30 minutes of assay completion.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells. Do not allow wells to dry.
14. Because Substrate Reagent is light sensitive, avoid prolonged exposure to light. Also avoid contact between Substrate Reagent and metal, or color may develop.

**SAFETY**

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

## DIRECTIONS FOR WASHING

**Incomplete washing will adversely affect the test outcome**. All washing must be performed with Wash Solution provided. The Wash Solution must be used at room temperature.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted Wash Solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, residual liquid is removed by inverting the plate and tapping on absorbent tissue. It is important that the wells are not allowed to dry.

Alternatively, the diluted Wash Solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with Wash Solution, completely filling all wells. After the washing procedure, residual liquid is removed by inverting the plate and tapping on absorbent tissue. It is important that the wells are not allowed to dry.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

## REAGENT PREPARATION AND STORAGE

### Wash Solution

Dilute 100 mL of Wash Solution Concentrate with 900 mL distilled water. Dissolve the Wash Solution completely when making the dilution. The diluted Wash Solution is stable for 2 weeks at 2 to 8°C. When Wash Solution is stored at 2 to 8°C, some turbidity may appear. This turbidity does not affect the assay results. Allow the Wash Solution to come to room temperature and mix to re-dissolve the precipitate.

### HRP-Conjugate Solution

The Conjugate Concentrate must be diluted just prior to use. Dilute the Conjugate Concentrate 1:101 with Conjugate Diluent. The diluted Conjugate is not stable. Dilute only the quantity required for the assay. Return the unused Conjugate Concentrate to the refrigerator after use.

### Preparation of Hu IL-18 Standard Curve

Reconstitute the Hu IL-18 Calibrator with the volume of Assay Diluent indicated in the Lot Specific Package Insert. The reconstituted Calibrator may be apportioned into working aliquots and stored at -20°C if storage is required. Avoid repeated freeze-thaw cycles. The standard curve should be prepared as indicated by the Lot Specific Package Insert.

## ASSAY METHOD: PROCEDURE AND CALCULATIONS

**Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.**

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

**Note**: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame for current use. (Re-bag extra strips. Store these in the refrigerator for future use.)
2. In order to obtain optimal precision, 150 µL of prepared standards and samples should be pipetted into a new 96-well polyvinyl plate in the same order as the assay is to be run. Once all samples and standards have been prearranged in the polyvinyl plate, transfer 100 µL of each sample to the antibody coated wells simultaneously using a multi-channel pipette. The reaction starts as soon as the samples are pipetted into the antibody coated wells; therefore, pipetting should be completed as quickly as possible.
3. Incubate 60 minutes at room temperature.
4. Aspirate or discard the contents of each well, then fill each well with Wash Solution. Aspirate or discard the contents. Wash each well 3 additional times. Aspirate or discard Wash Solution remaining in the wells. After the washing procedure, residual liquid is removed by inverting the plate and tapping on absorbent tissue. It is important that the wells are not allowed to dry. **(Note: The Wash Solution should be used at room temperature.)**

5. Pour diluted Conjugate Reagent into a reservoir. Pipette 100 µL of Conjugate Reagent into each well with a multi-channel pipette.
6. Incubate 60 minutes at room temperature.
7. Aspirate or discard the contents of each well, then fill each well with Wash Solution. Aspirate or discard the contents. Wash each well 3 additional times as in step 4. After the washing procedure, residual liquid is removed by inverting the plate and tapping onto absorbent tissue. It is important that the wells are not allowed to dry.
8. Pour the Substrate Reagent into a clean reservoir. Pipette 100 µL of Substrate Reagent into each well. (Note: Substrate Reagent should be used at room temperature. Substrate Reagent is easily oxidized by metal ions. Use a clean pipette tip and reservoir when dispensing Substrate Reagent. Any unused Substrate Reagent remaining in the reservoir should be discarded. Do not return unused Substrate Reagent to the bottle.)
9. Incubate at room temperature for 30 minutes. A blue color will appear in Hu IL-18 containing wells.
10. Pour Stop Solution in a clean reservoir. Pipette 100 µL Stop Solution into each well. Upon addition of Stop Solution, the color within the wells changes from blue to yellow.
11. Measure the absorbance of each well at 450 nm using a microtiter plate reader. If a dual wavelength plate reader is available, read the plate at 450 nm with a reference wavelength of 620 nm. The measurement should be made within 30 minutes of adding Stop Solution. The plate reader can be blanked using a well containing 100 µL Substrate Reagent plus 100 µL Stop Solution.

12. Plot the absorbances of the standards against the standard concentration on graph paper. Draw the best smooth curve through these points and construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
13. Read the Hu IL-18 concentrations for unknown samples and controls from the standard curve plotted in step 12. Samples producing signals higher than that of the highest standard (1000 pg/mL) should be diluted in Assay Buffer and reanalyzed, multiplying the concentration found by the appropriate dilution factor.

**LIMITATIONS OF THE PROCEDURE**

Do not extrapolate the standard curve beyond the 1000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >1000 pg/mL with Assay Buffer; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of Hu IL-18 degradation in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

**This kit is for research use only.**
**Not for human therapeutic or diagnostic use.**

## PERFORMANCE CHARACTERISTICS

### SENSITIVITY

Sensitivity was determined by assaying serially diluted Hu IL-18 Calibrator. The mean absorbance plus 2 standard deviations for Calibrator diluted to 6.25 pg/mL was lower than the mean absorbance minus 2 standard deviations for Calibrator diluted to 12.5 pg/mL. The minimal detectable dose is therefore 12.5 pg/mL.

RECOVERY

Recombinant Hu IL-18 was added to samples at different concentrations. The Hu IL-18 concentration of the serum samples was calculated as described in ASSAY METHOD.

Serum 1

| (A) Added Hu IL-18 (pg/mL) | Hu IL-18 concentration observed pg/mL | (B) Recovery (pg/mL) | (B/A) Recovery (%) |
|---|---|---|---|
| 0.0 | 940.8 | - | - |
| 684.9 | 1583.0 | 642.2 | 94 |
| 1346.9 | 2179.0 | 1238.2 | 92 |
| 2354.1 | 3528.7 | 2587.9 | 110 |

Serum 2

| (A) Added Hu IL-18 (pg/mL) | Hu IL-18 concentration observed pg/mL | (B) Recovery (pg/mL) | (B/A) Recovery (%) |
|---|---|---|---|
| 0.0 | 868.9 | - | - |
| 684.9 | 1418.6 | 549.8 | 80 |
| 1346.9 | 2035.9 | 1167.0 | 87 |
| 2354.1 | 3431.3 | 2564 | 109 |

Serum 3

| (A) Added Hu IL-18 (pg/mL) | Hu IL-18 concentration observed pg/mL | (B) Recovery (pg/mL) | (B/A) Recovery (%) |
|---|---|---|---|
| 0.0 | 629.3 | - | - |
| 684.9 | 1334.7 | 705.4 | 103 |
| 1346.9 | 1913.4 | 1284.1 | 95 |
| 2354.1 | 3282.3 | 2653.1 | 113 |

REPRODUCIBILITY

1. Intra-Assay

Intra-assay reproducibility was determined by measuring Hu IL-18 concentrations in sera eight times. Hu IL-18 concentrations of the serum samples were calculated as described in ASSAY METHOD.

| | Serum 1 | Serum 2 | Serum 3 | Serum 4 | Serum 5 |
|---|---|---|---|---|---|
| Mean (pg/mL) | 2765.50 | 600.69 | 345.48 | 136.13 | 69.74 |
| C.V. (%) | 5.03 | 4.93 | 10.80 | 5.61 | 9.92 |

2. Inter-Assay Precision

Inter-assay reproducibility was determined by six independent assays of five serum samples. Hu IL-18 concentrations of the serum samples were calculated as described in ASSAY METHOD.

| | Serum 1 | Serum 2 | Serum 3 | Serum 4 | Serum 5 |
|---|---|---|---|---|---|
| Mean (pg/mL) | 2621.10 | 773.20 | 615.13 | 236.43 | 160.08 |
| C.V. (%) | 10.07 | 8.54 | 5.21 | 7.60 | 6.25 |

## SPECIFICITY

Buffered solutions of a panel of substances at 1 ng/mL were assayed with the Invitrogen Hu IL-18 ELISA kit. The following substances were tested and found to produce results below the detection limit of 12.5 pg/mL: IFN-α, IFN-γ, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12, GM-CSF, and murine IL-18.

## EXPECTED VALUES

Serum samples from 46 normal healthy donors were assayed with the Invitrogen Hu IL-18 ELISA kit. The values ranged from 36.05-257.75 pg/mL. The mean value was 125.96 pg/mL. The standard deviation was 44.48. The mean plus 3 standard deviations was 259.41 pg/mL.

## REFERENCES


1. Okamura, H., et al. (1995) Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 378(6552):88-91.
2. Ushio, S., et al. (1996) Cloning of the cDNA for human IFN-gamma-inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. J. Immunol. 156(11):4274-4279.
3. Micallef, M.J., et al. (1996) Interferon-gamma-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-gamma production. Eur. J. Immunol. 26(7):1647-1651.
4. Dao, T., et al. (1996) Interferon-gamma-inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of murine T helper 1 cells. Cell. Immunol. 173(2):230-235.


Manufactured for Invitrogen by MBL.

**NOTES**