Human IL-6 ELISA Kit

to determine Human IL6 in Serum, Blood Plasma, Saliva, Urine, Tissue

Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT7106ETKKBA

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Toll Free: 1800-120-2434
Mobile: +91-98105-21400 | Fax: +91-11-42208444
customerservice@lifetechindia.com

INTENDED USE

This kit is used to determine Human IL6 in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.02

ASSAY SUMMARY

IL-6 gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, the encoded protein has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation. Human IL-6 ELISA Kit employs a two-site sandwich ELISA to quantitate Human IL-6 in samples. An antibody specific for Human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Human IL-6 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Human IL-6 is added to the wells. After washing, proprietary Streptavidin-HRP conjugate is added to the wells. Following a wash to remove any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Human IL-6 bound in the initial step. The color development is stopped by Stop Solution and the intensity of the color is measured.

CHARACTERISTICS

• This Kit allows for the determination of Human IL-6 concentrations in Human serum, Plasma, cell culture supernates and other biological fluids.

• Detection range: 3.13 pg/mL - 200 pg/mL.

• The minimum detectable dose (MDD) of Human IL-6 is typically less than 2 pg/mL.

• Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)＜10%.

• Three samples of known concentration were tested in twenty separate assays to inter-assay precision. Assays were performed by at least three technicians using two of components. The CV (%)＜12%.

• To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human IL-6 were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient 0.99.

• Human IL-6 ELISA Kit has high sensitivity and excellent specificity for detection of Human IL-6. No significant cross-reactivity or interference between Human IL-6 and analogues was observed.

• The recovery of Human IL-6 spiked to different levels in samples throughout the range the assay in various matrices was evaluated.

The recovery ranged from 98% to 116% with an overall mean recovery of 106%.

PRODUCT INFORMATION

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

Components	48T	96T	Storage Conditions
Human IL-6 microplate	48 well	96 wells	2-8°C
Human IL-6 standard	1 (lyophilized)	2 (lyophilized)	2-8°C
Sample Diluent	3.5 mL (5×)	7 mL (5×)	2-8°C
Assay Buffer	3.5 mL (5×)	7 mL (5×)	2-8°C
Human IL-6 Detect Antibody	60 µL (100×)	120 µL (100×)	2-8°C
Streptavidin-HRP	60 µL (100×)	120 µL (100×)	2-8°C
HRP Substrate (TMB)	5 mL	10 mL	2-8°C
Stop Solution	5 mL	10 mL	2-8°C
Wash Buffer	25 mL (20×)	50 mL (20×)	2-8°C
Plate Covers	1	2	RT
Booklet	1	1	RT

[1]Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at

approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Note: Samples should be centrifuged adequately and no hemolysis or granule was allowed.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

The dilution of Wash buffer and Sample Diluent/ Assay buffer in the following operation steps is based on 96T kit. If a 48T kit is used, please scale it down.

Wash Buffer - Pour entire contents (50 mL) of the Wash buffer (20×) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2 to 25˚C. Please note that Wash buffer (1×) is stable for 30 days.

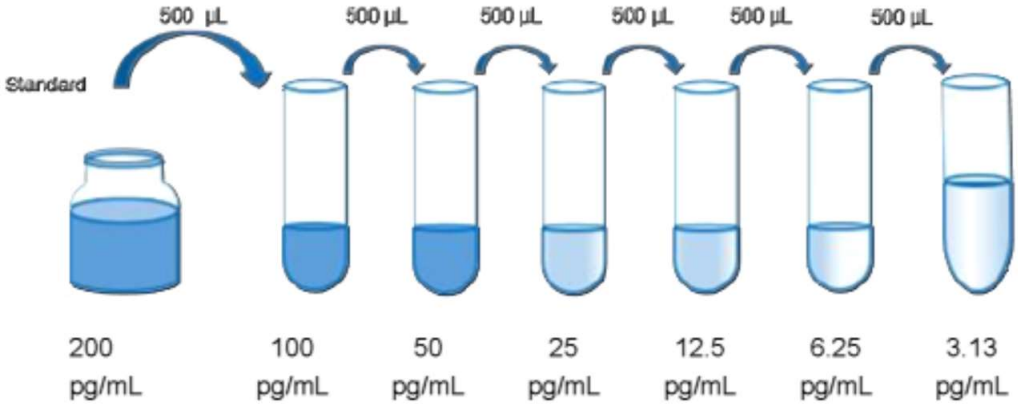
Sample Diluent/ Assay Buffer - Pour the entire contents (7 mL) of the Diluent (5×) into a clean 100 mL graduated cylinder. Bring to final volume of 35 mL with distilled water. Mix gently to avoid foaming. Store at 2 to 8˚C. Please note that the Diluent (1×) is stable for 30 days.

HRP Substrate (TMB) - The reagents should be ready within 15 minutes of use. Protect from light. 100 µL of the solution is required per well.

Standard - Reconstitute the Human IL-6 standard in 1 mL of Sample Diluent for a

concentration of 200 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Add 500 µL of Sample Diluent Buffer to each of 6 tubes labeled 100, 50, 25, 12.5, 6.25 and 3.13 pg/mL of Human IL-6 standard just as below.



Human IL-6 Detect Antibody - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with Assay buffer in a clean plastic tube as needed according to the standards and samples. Detect antibody should be used within 30 minutes after dilution.

Streptavidin-HRP - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Streptavidin-HRP with Assay buffer in a clean plastic tube as needed according to the standards and samples. Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Diluent - If your samples need to be diluted, Sample diluent is used for dilution of serum/plasma samples, while cell culture medium is used for dilution of cell culture supernates.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended

that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents and working standards 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 100 µL of diluted standard and sample per well. Add 100 µL Sample Diluent to Blank well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.

4. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or automatic washer. 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.

5. Add 100 µL of diluted Human IL-6 detect antibody to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.

6. Repeat the aspiration/wash as in step 4.

7. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.

8. Repeat the aspiration/wash process for five times as in step 4.

9. Add 100 µL of HRP substrate solution to each well. Incubate for 15 minutes at room temperature. Protect from light.

10. Add 50 µL of Stop solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Human IL-6 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

PRECAUTIONS

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing

technique, incubation time or temperature, and kit age can cause variation in binding.

4. When mixing or reconstituting protein solutions, always avoid foaming.
5. 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.
6. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
7. Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.
8. 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 blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. Liquid Waste: 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 the viruses before disposal.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).