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MaxLISA IL-6 ELISA Test Kit

Intended Use

The purpose of the MaxLISA IL-6 ELISA Test Kit is intend for the quantitative determination of human Interleukin 6 (IL-6) concentrations in cell culture supernates, serum, and plasma.

Order Information

Pack Size	Presentation

SUMMARY

Interleukin 6 (IL-6) is a pleiotropic, α-helical, 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression. Mature human IL-6 is 183 amino acids (aa) in length and shares 39% aa sequence identity with mouse and rat IL-6. Alternative splicing generates several isoforms with internal deletions, some of which exhibit antagonistic properties. Cells known to express IL-6 include CD8+ T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, B1 B cells and pancreatic islet beta cells. IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids. Normal human circulating IL-6 is in the 1 pg/mL range, with slight elevations during the menstrual cycle, modest elevations in certain cancers, and large elevations after surgery. IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6 R alpha) and a signal transducing subunit (gp130). IL-6 binds to IL-6 Rα, triggering IL-6 Rα association with gp130 and gp130 dimerization. gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM. Soluble forms of IL-6 Rα are generated by both alternative splicing and proteolytic cleavage. In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 Rα elicit responses from gp130-expressing cells that lack cell surface IL-6 Rα. Trans-signaling enables a wider range of cell types to respond to IL-6, as the expression of gp130 is ubiquitous, while that of IL-6 Rα is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes. Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 Rα but not from other cytokines that use gp130 as a co-receptor. IL-6, along with TNF-α and IL-1, drives the acute inflammatory response. IL-6 is almost solely responsible for fever and the acute phase response in the liver, and it is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease. When dysregulated, it contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, arthritis and sepsis. IL-6 modulates bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis through its promotion of Th17 cell development and activity. It contributes to atherosclerotic plaque development and destabilization as well as the development of inflammation-associated carcinogenesis. IL-6 can also function as an anti-inflammatory molecule, as in skeletal muscle where it is secreted in response to exercise. In addition, it enhances hematopoietic stem cell proliferation and the differentiation of memory B cells and plasma cells. The Human IL-6 ELISA Test Kit is a 1.5 hour solid phase immunoassay designed to measure human IL-6 in cell culture supernates, serum, and plasma. It contains E. coli-expressed recombinant human IL-6, and antibodies raised against the recombinant protein. Natural human IL-6 showed dose-response curves that were parallel to the standard curves obtained using the kit standards, indicating that this kit can be used to determine relative levels of natural human IL-6. For research use only. Not for use in diagnostic procedures. It has been observed in our laboratories that the measurement of IL-6 is insensitive to the addition of the recombinant form of the IL-6 soluble receptor. Therefore it is probable that experimental sample measurements reflect the total amount of IL-6 present,
i.e., the total amount of free IL-6 plus the amount of IL-6 initially bound to soluble receptors, if any are present in the samples. High levels of high-affinity autoantibodies to IL-6 in the serum of some blood donors have been reported. Such autoantibodies have the potential to interfere with the measurement of IL-6 by ELISA immunoassays.

PRINCIPLE OF THE TEST

This ELISA Test Kit employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped and the intensity of the color is measured.

MATERIALS AND COMPONENTS

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

MICROPLATE: Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains monoclonal antibody specific for human IL-6. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once open, stable for one month at 2-8°C.

CALIBRATOR: (1x1ml per vial). Colorless liquid filled in a vial with white screw cap. recombinant human IL-6 diluted in protein-stabilized buffer wih 0.1% ProClinTM 300. recombinant human IL-6 is 300pg/ml. Once open, stable for one month at 2-8°C. CALIBRATOR DILUENT: (1x6.0ml per bottle). Colorless liquid filled in a bottle with Green screw cap. Protein-stabilized buffer wih 0.1% **ProClin™** 300. Once open, stable for one month at 2-8°C.

CONJUGATE: (1x6ml per bottle) Protein-stabilized buffer wih 0.1% ProClinTM 300. Red-colored liquid in a white bottle with red screw cap. Horseradish peroxidase-conjugated mouse antibody to human IL-6. Ready to use as supplied. Once open, stable for one month at 2-8°C.

WASH BUFFER: (1x20ml per bottle).

DILUTE BEFORE USE! Detergent Tween-20 Colorless liquid filled in a white bottle with white screw cap, PH 7.4, 20 × PBS. The concentrate must be diluted 1 to 19 with distilled/deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks when stored at 2-8°C.

SUBSTRATE SOLUTION A: (1x6ml per bottle). Colorless liquid filled in a white bottle with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.

SUBSTRATE SOLUTION B: (1x6ml per bottle). Colorless liquid filled in a black bottle with black screw cap. TMB (Tetramethyl benzidine) solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.

STOP SOLUTION: (1x6ml per bottle) Colorless liquid in a white bottle with white screw cap. Diluted sulfuric acid solution (0.5M H2SO4).Ready to use as supplied. Once open, stable for one month at 2-8°C.

PLASTIC SEALABLE BAG: For enclosing the strips not in use

PACKAGE INSERT CARDBOARD PLATE COVER

To cover the plates during incubation and prevent evaporation or contamination of the wells

MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, ry incubator or water bath, 37±0.5°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, microwell aspiration/wash system.

SPECIMAN COLLECTION, TRANSPORTATION AND STORAGE

1. Specimen Collection normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the speci should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.

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2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric, hemolytic specimens or should not be used as they can give false results in the assay. Do not heat inactivate specimens This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. This ELISA is intended ONLY for testing of individual cell culture supernates, serum or plasma samples. Do no kit use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 7 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of this ELISA kit, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS The ELISA assay are time and temperature sensitive. To avoid incorrect result, ***strictly follow the test procedure steps and do not modify them.***

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes beyond their expiry date stated on labels or boxes.
3. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake the reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprint reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next ste formation of air bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin shoul Laboratory Practice) regulations can ensure the personal safety.
15. WARNING: There is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClinTM 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT: Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results substitute the reagents with new one.

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIE**

1. **Preparation:** Format the microplate's wells for control and patient specimen to be assayed. Replace any unused microwell strips back into the aluminum bag seal and store at 2-8°C.
2. Diluting Calibrator：The Calibrator in the kit is 300pg/ml, which is Calibrator F. Use Calibrator F to Prepare Calibrator E by being diluted 1:3 with Calibrator Diluent (Recommended adding 200µl Calibrator F into 400µl Calibrator Diluent and mixing). Add 200µl Calibrator E into 400µl Calibrator Diluent to prepare calibrator D, Add 200µl Calibrator D into 400µl Calibrator Diluent to prepare calibrator C and so on, Calibrator Diluent is Calibrator A. Six concentrations of Calibrators (A-F): A: 0, B: 3.7pg/ml, C: 11.1pg/ml, D: 33.3pg/ml, E: 100pg/ml and F: 300pg/ ml can be prepared
3. Adding Sample and Conjugate: Set 1 blank well, 6 calibrator wells (A-F); add 50µl of sample or calibrator into each well and then add 50µl of CONJUGATE (except blank well!). Mix by tapping the plate gently.
4. Incubating: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.
5. Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
6. Adding Substrate: Add 50µl of Substrate Solution A and 50µl of Substrate Solution B into each well. Incubate for 15 minutes at 37°C avoiding light.
7. Adding Stop Solution: Using a multichannel pipette or manually, add 50µl of Stop Solution into each well and mix gently.
8. Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP content of the wells but allow the plate washer to aspirate it automatica
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solutio concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1 to 20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

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QUALITY CONTROL AND CALCULATION OF THE RESULTS

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by each specimen (A) value to concentration of human IL-6. Using single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and calibrators. In case the reading is based in dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and calibrators.

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed

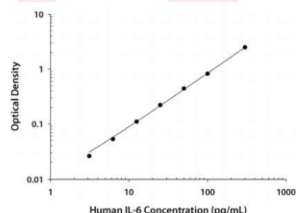
- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
- The A value of the Calibrator F must be ≥ 1.500 at 450/630nm or at 450nm after blanking.
- The A value of the Calibrator A must be < 0.200 at 450/630nm or at 450nm after blanking.

If any of quality control criteria do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

CALCULATION OF THE RESULTS

1. Calculate the average read relative absorbance value for each set of reference calibrators and samples.
2. We recommend using proper software to calculate the results. The best curve fitting used in the assays are quadratic regression or 4 by plotting the mean absorbance linear graph paper, with value on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of pg/ml from the standard curve. Example of a Standard Curve For illustration purpose only, the average values are given only:

Calibrator	Standard pg/ml	Mean OD
A	0	0.020
B	3.7	0.041
C	11.1	0.139
D	33.3	0.481
E	100	1.305
F	300	2.316



PERFORMANCE CHARACTERISTICS

Analytical Performance

Detection Region：0-300pg/ml

Detection CV value: < 10%

Limit of detection (LoD): 1pg/ml

No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.

No interference from rheumatoid factors: up to 2000U/ml observed.

No high dose hook effect: up to 10000pg/ml.

Frozen specimens have been tested too to check for interferences due to collection and storage.

LIMITATIONS

1. Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.
2. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
3. Use fresh samples whenever possible. Frozen and thawed samples (especially repeatedly) can lead to aberrant results.
4. Optimal assay performance requires strict adherence to the assay procedure described in this insert sheet. Deviations may lead to aberrant results.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

REFERENCES

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SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not lot- interchangeable.

1. Microplate	1x96wells
2. Calibrator	1x1ml
3. Calibrator Diluent	1x6ml
4. Conjugate	1x6ml
6. Wash Buffer	1x20ml
7. Substrate Solution A	1x6ml
8. Substrate Solution B	1x6ml
9. Stop Solution	1x6ml

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