



ClinMax™ Human IL-6 ELISA Kit, PRO

Catalog Number: CEA-C001

Pack Size: 96 tests

**IMPORTANT: Please carefully read this manual before performing your experiment.
For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure**

TABLE OF CONTENTS

| | |
|--|----|
| INTENDED USE | 1 |
| PRINCIPLE OF THE ASSAY | 1 |
| LIMITATIONS OF THE PROCEDURE | 2 |
| MATERIALS PROVIDED | 2 |
| REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED | 2 |
| KIT STORAGE AND EXPIRATION DATE | 3 |
| REAGENT PREPARATION | 3 |
| RECOMMENDED SAMPLE PREPARATION | 4 |
| CALCULATION OF RESULTS | 5 |
| PRECAUTIONS FOR USE | 6 |
| QUICK GUIDE | 7 |
| TYPICAL DATA | 8 |
| PERFORMANCE CHARACTERISTICS | 8 |
| TROUBLESHOOTING GUIDE | 14 |

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INTENDED USE

This kit is specifically designed for the accurate quantitation of human Interleukin 6 (IL-6) from serum and plasma. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

Interleukin-6 (IL-6) is a member of the pro-inflammatory cytokine family. It induces the expression of a variety of proteins responsible for acute inflammation, and plays an important role in the proliferation and differentiation of cells in humans. It has been demonstrated the dysregulation of the IL-6 signaling pathways can result in inflammatory and autoimmune disorders as well as cancer development.

Human IL-6 (also known as B-cell stimulatory factor 2, Interferon beta-2, Hybridoma growth factor, and CTL differentiation factor) plays an essential role in the final differentiation of B-cells into Ig-secreting cells. It induces myeloma and plasmacytoma growth, nerve cells differentiation, and acute phase reactants in hepatocytes. IL-6 is expressed by T cells, B cells, monocytes, fibroblasts, hepatocytes, endothelial cells, and keratinocytes. It has been shown that IL-6 plays a critical role in many physiological and pathological conditions, including autoimmune diseases and rheumatoid arthritis.

This assay kit is used to measure the concentrations of human Interleukin 6 (IL-6) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti- IL-6 Antibody. First add the standard samples provided in kit and your samples to the plate, next add detection antibody Biotin-Anti-IL-6 Antibody to the plate, incubate and wash the wells. After wash add HRP-Streptavidin to the plate, incubate and wash the wells. Lastly load the substrate into the wells and color develops in proportion to the amount of Interleukin 6 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450nm and 630nm.

LIMITATIONS OF THE PROCEDURE

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.

MATERIALS PROVIDED

Table 1. Materials provided

| Catalog | Components | Size (96 tests) | Format | Storage | |
|------------|------------------------------------|--------------------|--------------------|----------|---------|
| | | | | Unopened | Opened |
| CEA001-C01 | Pre-coated Anti-IL-6 Ab Microplate | 1 plate | Solid | 2-8 °C | 2-8 °C |
| CEA001-C02 | IL- 6 Calibrator | 20µg ×2 | Lyophilized powder | 2-8 °C | -70 °C |
| CEA001-C03 | Biotin-Anti-IL- 6 Ab Con. Solution | 100 µL | Liquid | 2-8 °C | 2-8 °C |
| CEA001-C04 | Biotin-Ab Dilution Buffer | 8 mL | Liquid | 2-8 °C | 2-8 °C |
| CEA001-C05 | IL-6 SA-HRP Con. Solution | 0.5 mL | Liquid | 2-8 °C | 2-8 °C |
| CEA001-C06 | SA-HRP Dilution Buffer | 15 mL | Liquid | 2-8 °C | 2-8 °C |
| CEA001-C07 | 20× Washing Buffer | 50 mL | Liquid | 2-30 °C | 2-30 °C |
| CEA001-C08 | Sample Dilution Buffer | 15 mL ×2 | Liquid | 2-8 °C | 2-8 °C |
| CEA001-C09 | Substrate Solution | 12 mL | Liquid | 2-8 °C | 2-8 °C |
| CEA001-C10 | Stop Solution | 6 mL | Liquid | 2-30 °C | 2-30 °C |

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Centrifuge;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized water / ultrapure water / distilled water to dilute 20× Washing Buffer.

SAMPLE COLLECTION & STORAGE

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

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g within 30 minutes of collection. Assay immediately.

Storage - Samples which cannot be assayed within 24 hours of collection should be frozen at -20°C or lower and will be stable for up to six months. Samples are allowed to freeze-thaw once.

KIT STORAGE AND EXPIRATION DATE

The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.

The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (18-25 °C) before use. If crystals have formed in buffer solution, place the sample in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, and the size of the aliquot should not be less than 10 µg.

Table 2. Preparation method

| Catalog | Components | Size (96 tests) | Storage solution concentration | Reconstituted water volume |
|------------|-----------------|--------------------|--------------------------------|----------------------------|
| CEA001-C02 | IL-6 Calibrator | 20 µg | 20 µg/mL | 1 mL |

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer

Dilute 50 mL 20×Washing Buffer with deionized water / ultrapure water / distilled water to 1000 mL.

1.2 Preparation of Biotin-Anti-IL-6 Ab Solution

Prepare Biotin-Anti-IL-6 Ab Solution by diluting 30 µL of Biotin-Anti-IL-6 Ab Con. Solution into 6 mL Biotin-Ab Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

1.3 Preparation of IL-6 SA-HRP Solution

Prepare IL-6 SA-HRP Solution by diluting 240 µL of IL-6 SA-HRP Con. Solution into 12 mL SA-HRP Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

2. Preparation of Calibration curve

The concentration of the reconstituted human IL-6 Calibrator (CEA001-C02) is 20 µg/mL, prepare Cm by diluting 5 µL of the reconstituted human IL-6 Calibrator into 995 µL Sample Dilution Buffer, mix gently well. Label 7 tubes, one for each standard point: C1, C2, C3, C4, C5, C6, C7. According to the following dilution scheme: 4 µL IL-6 Cm + 996 µL Sample Dilution Buffer. Shake gently to mix, labeled C1 (C1 = 400 pg/mL). Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 500 µL of Sample Dilution Buffer into each tube. Pipette 500 µL of diluted standard (concentration of standard = 400 pg/mL) into the first tube, labeled C2 (C2 = 200 pg/mL), and mix. Pipette 500 µL of this dilution into the second tube, labeled C3, and mix thoroughly before the next transfer. Sample Dilution Buffer serves as blank.

3. Add Samples and Biotin-Ab Solution

Add 50 µL IL-6 Calibrator to each well, or add 50 µL samples to each well, finally add 50 µL Biotin-Anti-IL-6 Ab Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 1.0 h.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for five times.

5. Add IL-6 SA-HRP Solution

For all wells, add 100 µL IL-6 SA-HRP Solution. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 30 min, avoid light.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 15 min, avoid light.

8. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: the color in the wells should change from blue to yellow.

9. Data Recording

Read the absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

Note: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.

CALCULATION OF RESULTS

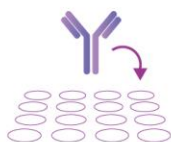
1. Normal range of Standard curve: $R^2 \geq 0.9900$.
2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.
3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Log-Log Linear regression equation are used to draw the standard curve and calculate the sample concentration.

PRECAUTIONS FOR USE

1. All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
2. Do not use kit reagents beyond expiration date on label.
3. In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
4. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
5. Glass-distilled water or deionized water must be used for reagent preparation.
6. This kit should be used according to the provided instructions.
7. Do not mix reagents from different lots.
8. Bring all reagents and samples to room temperature (18-25 °C) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
9. This kit should be stored at 2-8 °C.

QUICK GUIDE

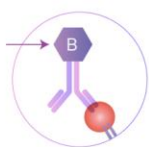
Quick Guide



1

Prepare

Prepare all reagents, standard curve, and samples as instructed.



2

Sample & detection antibody

Add test sample mix to wells. (Calibrator、samples、 Biotin-Ab Solution)



18-25°C 1.0 hour

Remove liquid and wash plate



3

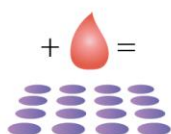
Streptavidin-HRP Solution

Add enzyme conjugated Streptavidin



18-25°C 30 min avoid light

Remove liquid and wash plate



4

Substrate Reaction

Colorimetric substrate is added to the wells and will form a colored solution when catalyzed by the enzyme.



18-25°C 15 min avoid light



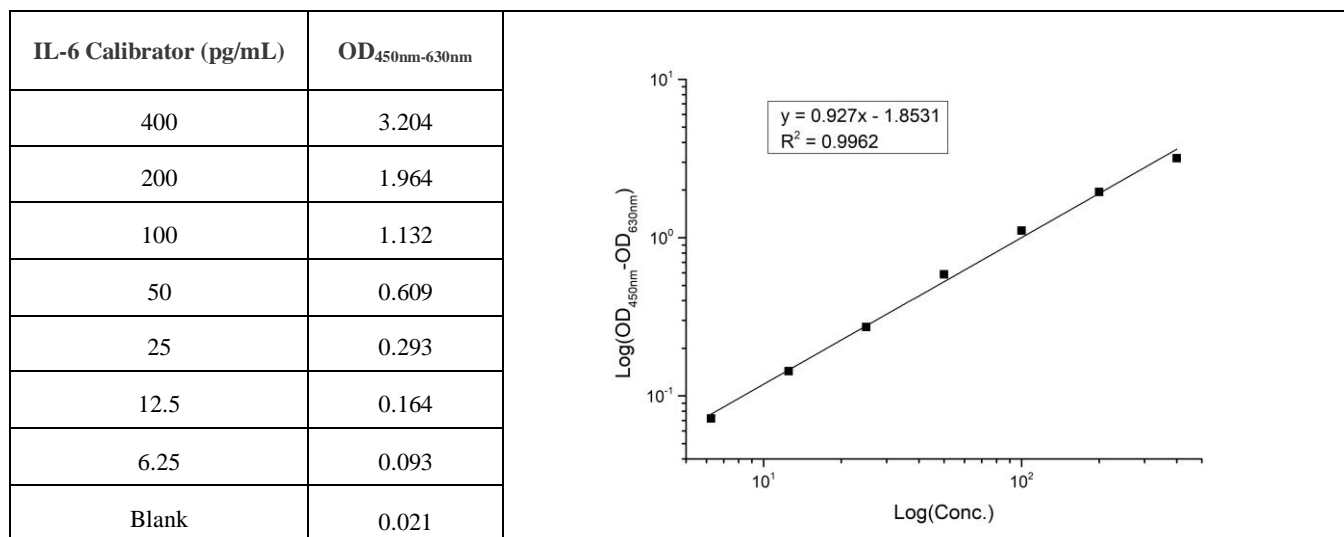
5

Termination +Analysis

Add Stop solution and read absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

TYPICAL DATA

Note: The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.



PERFORMANCE CHARACTERISTICS

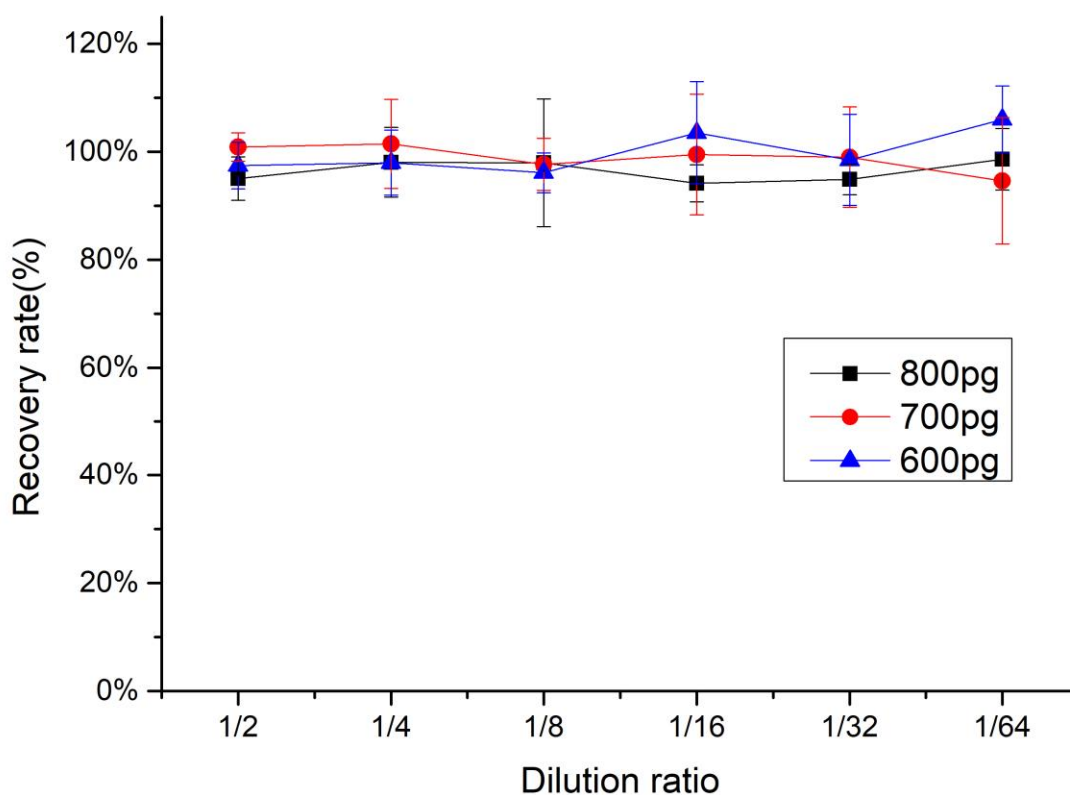
1. Sensitivity

The minimum detectable concentration of IL-6 is less than 1.0 pg/mL.

| | Lot 1 | Lot 2 | Lot 3 |
|------------------------|-------|-------|-------|
| Number of Replicates | 20 | 20 | 20 |
| Limit of blank (pg/mL) | 0.08 | 0.15 | 0.13 |

2. Linearity

Three human serum samples with high concentrations of IL-6 were diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 with Dilution Buffer to produce samples with values within the dynamic range and then assayed. On average, 98.43% of IL-6 was detected from serum samples.



3. Calibration

This immunoassay has been calibrated against a highly purified human IL-6 and is evaluated with standard from NIBSC/WHO. Reference Reagent Interleukin-6 (Human, rDNA derived) NIBSC code: 89/548.

NIBSC (89/548) approximate value (IU/mL) = $0.1 \times \text{ClinMax}^{\text{TM}}$ value (pg/mL).

4. Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 1 µg/mL.

| | |
|-------|--|
| Human | IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12 p70, IL-13, IL-15, IL-17, GM-CSF, TNF- α , IFN- γ |
|-------|--|

5. Intra-Assay Statistics

Ten replicates of each of five samples containing different IL-6 concentrations were tested in one assay , Intra-Assay Precision CV<10%.

| Lot 1 | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------------------|----------|----------|----------|----------|----------|
| Number of Replicates | 10 | 10 | 10 | 10 | 10 |
| Mean Concentration (pg/mL) | 353.30 | 279.84 | 171.77 | 12.53 | 3.79 |
| Standard Deviation | 24.60 | 17.38 | 10.04 | 0.88 | 0.34 |
| Coefficient of Variation (%) | 6.96% | 6.21% | 5.84% | 7.01% | 8.85% |
| Lot 2 | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
| Number of Replicates | 10 | 10 | 10 | 10 | 10 |
| Mean Concentration (pg/mL) | 302.12 | 238.49 | 172.50 | 12.20 | 3.57 |
| Standard Deviation | 9.28 | 10.18 | 5.95 | 0.45 | 0.17 |
| Coefficient of Variation (%) | 3.07% | 4.27% | 3.45% | 3.70% | 4.77% |
| Lot 3 | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
| Number of Replicates | 10 | 10 | 10 | 10 | 10 |
| Mean Concentration (pg/mL) | 325.98 | 254.86 | 174.96 | 11.32 | 3.07 |
| Standard Deviation | 7.06 | 15.32 | 9.40 | 0.64 | 0.19 |
| Coefficient of Variation (%) | 2.16% | 6.01% | 5.37% | 5.61% | 6.08% |

6. Inter-Assay Statistics

Five samples containing different concentrations of IL-6 were tested in three independent assays, Inter-Assay Precision CV<15%.

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------------------|----------|----------|----------|----------|----------|
| Number of Assays | 3 | 3 | 3 | 3 | 3 |
| Mean Concentration (pg/mL) | 327.14 | 257.73 | 173.08 | 12.02 | 3.48 |
| Standard Deviation | 26.12049 | 22.31012 | 8.463436 | 0.83424 | 0.385105 |
| Coefficient of Variation (%) | 7.98% | 8.66% | 4.89% | 6.94% | 11.07% |

7. Recovery

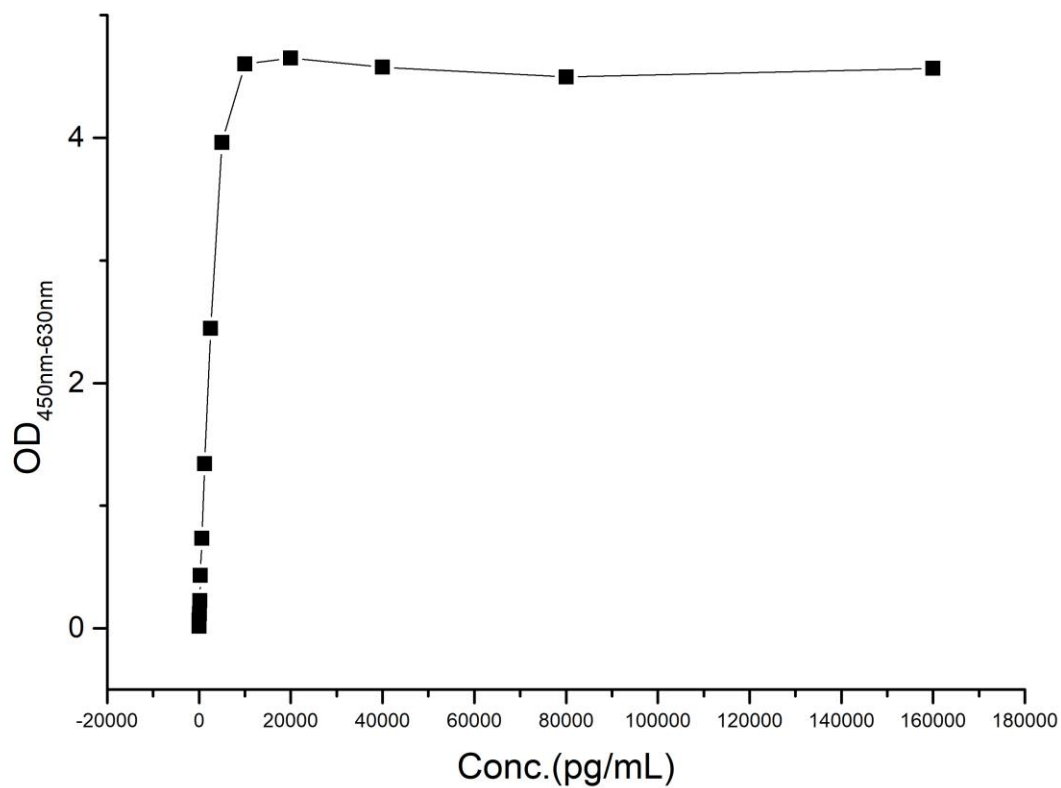
Recombinant IL-6 was spiked into 5 human serum samples, and then analyzed. On average, 100.58% of IL-6 was recovered from serum samples.

| | Lot 1 | | | | | Lot 2 | | | | | Lot 3 | | | | |
|-------------------------------------|---------|---------|--------|---------|---------|---------|---------|--------|--------|---------|--------|---------|---------|---------|---------|
| Sample Number | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Spiked Volume | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 |
| Sample volume | 4.13 | 3.55 | 4.98 | 3.67 | 2.24 | 4.19 | 3.32 | 5.22 | 4.03 | 2.39 | 4.22 | 2.99 | 4.83 | 4.31 | 2.28 |
| Sample Concentration (pg/mL) | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 |
| Spiked Concentration(pg/ml) | 97.94 | 113.71 | 104.20 | 102.77 | 114.58 | 90.08 | 101.87 | 99.76 | 102.94 | 104.78 | 89.75 | 109.32 | 107.47 | 109.71 | 106.08 |
| Spiked Sample Concentration (pg/mL) | 94.01% | 110.34% | 99.47% | 99.28% | 112.45% | 86.10% | 98.72% | 94.80% | 99.12% | 102.51% | 85.74% | 106.48% | 102.88% | 105.61% | 103.92% |
| Recovery rate (%) | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 |
| Spiked Concentration (pg/mL) | 83.96 | 84.84 | 70.63 | 76.37 | 69.51 | 80.45 | 80.87 | 65.21 | 66.56 | 70.77 | 68.45 | 77.84 | 69.35 | 84.11 | 86.89 |
| Spiked Sample Concentration (pg/mL) | 110.76% | 111.72% | 92.92% | 100.57% | 91.25% | 106.17% | 106.58% | 85.74% | 87.49% | 93.06% | 90.18% | 102.44% | 91.16% | 110.81% | 114.54% |
| Recovery rate (%) | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

| | | | | | | | | | | | | | | | |
|---|-------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Spiked Concentration (pg/mL) | 47.61 | 58.22 | 45.89 | 48.00 | 48.08 | 54.44 | 57.25 | 54.74 | 44.06 | 47.82 | 54.46 | 46.73 | 54.09 | 53.75 | 56.08 |
| Spiked Sample Concentration (pg/mL) | 93.12 | 114.32 | 90.01 | 94.09 | 94.42 | 106.8 | 112.4 | 107.8 | 86.46 | 93.88 | 107.2 | 91.51 | 106.4 | 105.3 | 109.9 |
| Recovery rate (%) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

8. Hook Effect

Not be affected by IL-6 concentrations up to 10 ng/mL.



9. Interference effect

Bilirubin (simulated jaundice) concentration should be less than 20mg/dL, hemoglobin (simulated hemolysis) concentration should not be higher than 3500mg/dL, triglyceride (simulated lipemia) concentration may not be higher than 2.0g/L, EDTA concentration should be less than 4mg/mL, and Sodium citrate concentration may not be higher than 40mg/mL, it does not affect the detection result.

| | Bilirubin (20mg/dL) | Hemoglobin (3500mg/dL) | Triglyceride (2.0g/L) | EDTA Plasma (4mg/mL) | Sodium citrate Plasma (40mg/mL) |
|--|--------------------------------|-----------------------------------|----------------------------------|---------------------------------|--|
| Spiked Sample Concentration (pg/mL)-1 | 9.88 | 9.85 | 10.70 | 9.76 | 8.72 |
| Sample Concentration (pg/mL)-1 | 9.57 | 9.57 | 9.57 | 9.57 | 9.57 |
| RD | 3.30% | 2.97% | 11.90% | 1.98% | -8.85% |
| Spiked Sample Concentration (pg/mL)-2 | 2.36 | 2.30 | 2.44 | 2.39 | 2.30 |
| Sample Concentration (pg/mL)-2 | 2.29 | 2.29 | 2.29 | 2.29 | 2.29 |
| Results | ≤Lob | ≤Lob | ≤Lob | ≤Lob | ≤Lob |

10. Sample values

240 human healthy sample were evaluated for the presence of human IL-6 in this assay. 53 samples measured less than the lowest standard, 5 pg/mL. 187 samples measured between 5 and 400 pg/mL.

TROUBLESHOOTING GUIDE

| Problem | Cause | Solution |
|---|--|--|
| Poor standard curve | * Inaccurate pipetting | * Check pipettes |
| Large CV | * Inaccurate pipetting * Air bubbles in wells | * Check pipettes * Remove bubbles in wells |
| High background | * Plate is insufficiently washed * Contaminated wash buffer | * Review the manual for proper wash. * Make fresh wash buffer |
| Very low readings across the plate | * Incorrect wavelengths * Insufficient development time | * Check filters/reader * Increase development time |
| Samples are reading too high, but standard curve looks fine | * Samples contain cytokine levels above assay range | * Dilute samples and run again |
| Drift | * Interrupted assay set-up * Reagents not at room temperature | * Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts |