Human Interleukin-6 (hIL-6) ELISA Kit

Catalog No.:  
KHC0061 (one plate kit; 96 determinations)  
KHC0062 (two plate kit; 192 determinations)  
KHC0061C (five plate kit; 480 determinations)

Sensitivity: <2 pg/mL  
Range: 7.8 - 500 pg/mL  
Calibration: The standard is calibrated against WHO reference preparation 89/548 (NIBSC, Hertfordshire, UK EN6 3QG); 1 pg = 100 mIU

Incubation Time: 3 hours at room temperature

Simple: Pre-coated strip-well plates  
Liquid stable conjugate, chromogen and stop reagents  
Sample size: 100 µL

Accurate:  
Inter-assay CV: ≤9.3%  
Intra-assay CV: ≤7.7%

Economical: One, two, or five 96-well plates, plus reagents for 96, 192, or 480 determinations. Reagents are stable for multiple runs.

Versatile: Serum, plasma (EDTA), cell culture medium or buffered solutions.

Species Reactivity: Lozier et al. (1999) cite the use of this kit’s antibody format in the measurement of IL-6 from rhesus monkey. Shean et al. (2000) cite the use of this kit in the measurement of IL-6 from rhesus monkey. Hensley et al. (2002) cite the use of this kit with rhesus monkey and cynomolgus monkey. Bilbo et al. (2002) report that this kit is cross-reactive with Siberian hamster.

**Human IL-6 ELISA Kit Summary Protocol**

1. Add 100 µL standards, specimens or controls to appropriate wells.  
2. Add 50 µL antigen-specific Biotin Conjugate to each well.  
3. Incubate wells at room temperature for 2 hours.  
4. Aspirate and wash wells 4x with Working Wash Buffer.  
5. Add 100 µL Streptavidin-HRP Working Conjugate to each well.  
6. Incubate for 30 minutes at room temperature.  
7. Aspirate and wash wells 4x with Working Wash Buffer.  
8. Add 100 µL of Stabilized Chromogen to each well.  
9. Incubate at room temperature for 30 minutes in the dark.  
10. Add 100 µL of Stop Solution to each well.  
11. Read absorbance at 450 nm.
References:


Yumoto, H., N. Nakae, K. Fujiinaka, S. Ebisu, and T. Matsuo (2001) Interleukin-6 (IL-6) and IL-8 are induced in human oral epithelial cells in response to exposure to periodontopathic Eikenella corrodens. Infection and Immunity January 384-394.


Immunoassay Kit
Catalog #KHC0061: 1 plate
    KHC0062: 2 plates
    KHC0061C: 5 plates

Human
IL-6

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</tbody>
</table>
INTRODUCTION

Human Interleukin 6 (IL-6) is a 184 A.A. polypeptide with potential O- and N-glycosylation sites and a significant homology with G-CSF. It is produced by various cells, including T- and B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, bone marrow stroma cells and several tumor cells. IL-6 regulates the growth and differentiation of various cell types with major activities on the immune system, hematopoiesis, and inflammation. These multiple actions are integrated within a complex cytokine network, where several cytokines induce (IL-1, TNF, PDGF, IFNs, etc.) or are induced by IL-6 and the final effects result from either synergistic or antagonistic activities between IL-6 and the other cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IFN-γ, GM-CSF, M-CSF, CSF, etc.). IL-6 induces final maturation of B-cells into antibody producing cells and is a potent growth factor for myeloma/plasmacytoma cells. It (co-)stimulates T-cell growth and cytotoxic T-cell differentiation. It promotes megakaryocyte development and synergizes with other cytokines to stimulate multipotent hematopoietic progenitors. IL-6 can also induce differentiation and growth inhibition of some leukemia or non-hematopoietic tumor cell lines. IL-6 is also a major inducer of the acute phase reactions in response to inflammation or tissue injury. Along with IL-1 and TNF, it induces the synthesis of acute phase proteins (APP) by hepatocytes, each cytokine or combination of cytokines showing a preferential pattern of APP production. IL-6 also interacts with the neuroendocrine system, e.g., by inducing ACTH production. Thus, IL-6 is a pleiotropic cytokine with multiple endocrine, paracrine and possibly autocrine activities in various tissues. Although most healthy individuals have undetectable levels of IL-6 in their serum, huge quantities of IL-6 are detected in severe inflammatory situations.
such as septicemia. The elevation of serum IL-6 precedes that of acute phase proteins, e.g., in a postoperative phenomenon, and may thus be a sensitive early parameter to investigate inflammatory conditions. Serum IL-6 has already been described in association with surgical or traumatic tissue injuries, infectious diseases, auto-immune diseases including arthritis, graft rejection, alcoholic liver cirrhosis, malignancies, etc.

**INTENDED USE**

The BioSource International, Inc. Human Interleukin-6 (hIL-6) ELISA is to be used for the *in vitro* quantitative determination of hIL-6 in human serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant hIL-6.

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 BioSource International, Inc. hIL-6 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for hIL-6 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known hIL-6 content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody.

During the first incubation, the hIL-6 antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of hIL-6 present in the original specimen.
**REAGENTS PROVIDED**

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>96 Test Kit</th>
<th>192 Test Kit</th>
<th>480 Test Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>hIL-6 Standard, recombinant hIL-6. Refer to vial label for quantity and reconstitution volume.</td>
<td>2 vials</td>
<td>4 vials</td>
<td>10 vials</td>
</tr>
<tr>
<td>Standard Diluent Buffer. Contains 7.7 mM sodium azide; 25 mL per bottle.</td>
<td>1 bottle</td>
<td>2 bottles</td>
<td>5 bottles</td>
</tr>
<tr>
<td>hIL-6 Antibody-Coated Wells, 96 wells per plate.</td>
<td>1 plate</td>
<td>2 plates</td>
<td>5 plates</td>
</tr>
<tr>
<td>hIL-6 Biotin Conjugate (Biotin-labeled anti-hIL-6). Contains 7.7 mM sodium azide; 6 mL per bottle.</td>
<td>1 bottle</td>
<td>2 bottles</td>
<td>5 bottles</td>
</tr>
<tr>
<td>Streptavidin-Peroxidase (HRP), (100x) concentrate. Contains 0.04% Proclin® 300, 3.3 mM thymol, and 0.06% methoxyphenol; 0.125 mL per vial.</td>
<td>1 vial</td>
<td>2 vials</td>
<td>5 vials</td>
</tr>
<tr>
<td>Streptavidin-Peroxidase (HRP) Diluent. Contains 0.04% Proclin® 300; 25 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>3 bottles</td>
</tr>
<tr>
<td>Wash Buffer Concentrate (25x); 100 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>2 bottles</td>
</tr>
<tr>
<td>Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>3 bottles</td>
</tr>
<tr>
<td>Stop Solution; 25 mL per bottle.</td>
<td>1 bottle</td>
<td>1 bottle</td>
<td>3 bottles</td>
</tr>
<tr>
<td>Plate Covers, adhesive strips.</td>
<td>3</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>
**Disposal Note:** This kit contains materials with small quantities of sodium azide and Proclin® 300. 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. Proclin® 300 is toxic. 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 - NOT PROVIDED**

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Deionized or distilled H₂O.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

**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 foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 - 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are >500 pg/mL should be diluted with Standard Diluent Buffer.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots.**
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. 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.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* 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 infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Buffer provided.

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, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

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

A. Reconstitution and Dilution of hIL-6 Standard

This assay has been calibrated against the WHO reference preparation 89/548 (NIBSC, Hertfordshire, UK, EN6 3QG). One pg equals 100 mIU.

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 2500 pg/mL with Standard Diluent Buffer. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.

2. Add 0.200 mL of the reconstituted standard to a tube containing 0.800 mL Standard Diluent Buffer. Label as 500 pg/mL hIL-6. Mix.

3. Add 0.300 mL of Standard Diluent Buffer to each of 6 tubes labeled 250, 125, 62.5, 31.2, 15.6, and 7.8 pg/mL hIL-6.

4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.
## B. Dilution of hIL-6 Standard

<table>
<thead>
<tr>
<th>Standard:</th>
<th>Add:</th>
<th>Into:</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 pg/mL</td>
<td>Prepare as described in Step 2.</td>
<td>0.300 mL of the Diluent Buffer</td>
</tr>
<tr>
<td>250 pg/mL</td>
<td>0.300 mL of the 500 pg/mL std.</td>
<td>0.300 mL of the Diluent Buffer</td>
</tr>
<tr>
<td>125 pg/mL</td>
<td>0.300 mL of the 250 pg/mL std.</td>
<td>0.300 mL of the Diluent Buffer</td>
</tr>
<tr>
<td>62.5 pg/mL</td>
<td>0.300 mL of the 125 pg/mL std.</td>
<td>0.300 mL of the Diluent Buffer</td>
</tr>
<tr>
<td>31.2 pg/mL</td>
<td>0.300 mL of the 62.5 pg/mL std.</td>
<td>0.300 mL of the Diluent Buffer</td>
</tr>
<tr>
<td>15.6 pg/mL</td>
<td>0.300 mL of the 31.2 pg/mL std.</td>
<td>0.300 mL of the Diluent Buffer</td>
</tr>
<tr>
<td>7.8 pg/mL</td>
<td>0.300 mL of the 15.6 pg/mL std.</td>
<td>0.300 mL of the Diluent Buffer</td>
</tr>
<tr>
<td>0 pg/mL</td>
<td>0.300 mL of the Diluent Buffer</td>
<td>An empty tube</td>
</tr>
</tbody>
</table>

Discard all remaining reconstituted and diluted standards after completing assay. Return the Standard Diluent Buffer to the refrigerator.
C. Storage and Final Dilution of Streptavidin-HRP

Please Note: The Streptavidin-HRP 100x concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow Streptavidin-HRP concentrate to reach room temperature. Gently mix. Pipette Streptavidin-HRP concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 μL of this 100x concentrated solution with 1 mL of Streptavidin-HRP Diluent for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

<table>
<thead>
<tr>
<th># of 8-Well Strips</th>
<th>Volume of Streptavidin-HRP Concentrate</th>
<th>Volume of Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20 μL solution</td>
<td>2 mL</td>
</tr>
<tr>
<td>4</td>
<td>40 μL solution</td>
<td>4 mL</td>
</tr>
<tr>
<td>6</td>
<td>60 μL solution</td>
<td>6 mL</td>
</tr>
<tr>
<td>8</td>
<td>80 μL solution</td>
<td>8 mL</td>
</tr>
<tr>
<td>10</td>
<td>100 μL solution</td>
<td>10 mL</td>
</tr>
<tr>
<td>12</td>
<td>120 μL solution</td>
<td>12 mL</td>
</tr>
</tbody>
</table>

2. Return the unused Streptavidin-HRP concentrate to the refrigerator.
D.  Dilution of Wash Buffer

Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

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(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)

2. Add 100 μL of the Standard Diluent Buffer to zero wells. Well(s) reserved for chromogen blank should be left empty.

3. Add 100 μL of standards, samples or controls to the appropriate microtiter wells. (See REAGENT PREPARATION AND STORAGE, Section B.)
4. Pipette 50 μL of biotinylated anti-IL-6 (Biotin Conjugate) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.

5. Cover plate with plate cover and incubate for 2 hours at room temperature.

6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.

7. Add 100 μL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in REAGENT PREPARATION AND STORAGE, Section C.)

8. Cover plate with the plate cover and incubate for 30 minutes at room temperature.

9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.

10. Add 100 μL of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue.

11. Incubate for 30 minutes at room temperature and in the dark. **Please Note:** Do not cover the plate with aluminum foil or metalized mylar. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the Stop Solution has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
12. Add 100 μL of Stop Solution to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 μL each of Stabilized Chromogen and Stop Solution. Read the plate within 2 hours after adding the Stop Solution.
14. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
15. Read the hIL-6 concentrations for unknown samples and controls from the standard curve plotted in Step 14. (Samples producing signals greater than that of the highest standard (500 pg/mL) should be diluted in Standard Diluent Buffer and re-analyzed, multiplying the concentration found by the appropriate dilution factor.)
**TYPICAL DATA**

The following data were obtained for the various standards over the range of 0 to 500 pg/mL hIL-6.

<table>
<thead>
<tr>
<th>Standard hIL-6 (pg/mL)</th>
<th>Optical Density (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>0.060</td>
</tr>
<tr>
<td>7.8</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>0.100</td>
</tr>
<tr>
<td>15.6</td>
<td>0.138</td>
</tr>
<tr>
<td></td>
<td>0.132</td>
</tr>
<tr>
<td>31.2</td>
<td>0.228</td>
</tr>
<tr>
<td></td>
<td>0.242</td>
</tr>
<tr>
<td>62.5</td>
<td>0.390</td>
</tr>
<tr>
<td></td>
<td>0.404</td>
</tr>
<tr>
<td>125</td>
<td>0.720</td>
</tr>
<tr>
<td></td>
<td>0.734</td>
</tr>
<tr>
<td>250</td>
<td>1.325</td>
</tr>
<tr>
<td></td>
<td>1.335</td>
</tr>
<tr>
<td>500</td>
<td>2.518</td>
</tr>
<tr>
<td></td>
<td>2.668</td>
</tr>
</tbody>
</table>
LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 500 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >500 pg/mL with Standard Diluent Buffer; re-analyze 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 degradation of native hIL-6 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

The minimum detectable dose of hIL-6 is <2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 32 times.
PRECISION

1. Intra-Assay Precision

Serum-based and buffer-based samples of known hIL-6 concentration were assayed in replicates of 16 to determine precision within an assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/mL)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>38.8</td>
<td>3.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Sample 2</td>
<td>101.2</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Sample 3</td>
<td>242.7</td>
<td>12.5</td>
<td>5.1</td>
</tr>
</tbody>
</table>

\[SD = \text{Standard Deviation}\]
\[CV = \text{Coefficient of Variation}\]

2. Inter-Assay Precision

Samples were assayed 8 times in 5 different assays to determine precision between assays.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/mL)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>35.3</td>
<td>3.3</td>
<td>9.3</td>
</tr>
<tr>
<td>Sample 2</td>
<td>97.4</td>
<td>6.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Sample 3</td>
<td>236.7</td>
<td>18.5</td>
<td>7.8</td>
</tr>
</tbody>
</table>

\[SD = \text{Standard Deviation}\]
\[CV = \text{Coefficient of Variation}\]
LINEARITY OF DILUTION

Human serum containing 222 pg/mL of measured hIL-6 was serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.998.

RECOVERY

The recovery of hIL-6 added to normal human serum and normal human plasma averaged 88% and 70%, respectively. The recovery of hIL-6 added to tissue culture medium containing 10% fetal calf serum averaged 111%.

SPECIFICITY

Buffered solutions of a panel of substances at 50 ng/mL were assayed with the BioSource International, Inc. hIL-6 kit. The following substances were tested and found to have no cross-reactivity: human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-7, IL-8, IL-10, G-CSF, GM-CSF, IFN-α, IFN-γ, LIF, MIP-1α, MIP-1β, MCP-1, OSM, RANTES TGF-β, TNF-α, TNF-β; mouse IL-6; rat IL-6.

EXPECTED VALUES

Eight samples, that consisted of 10% human whole blood in culture medium stimulated with LPS/PHA, were assayed for hIL-6. The values ranged from 720-12,000 pg/mL. The mean value was 2500 pg/mL.
REFERENCES

Human IL-6 Assay Summary

Add 100 μL of standards, controls & samples

Add 50 μL of Biotin Conjugate
Incubate for 2 hours at RT
↓ aspirate and wash 4x

Incubate 100 μL of Streptavidin-HRP Working Solution for 30 minutes at RT
↓ aspirate and wash 4x

Incubate 100 μL of Stabilized Chromogen for 30 minutes at RT
↓

Add 100 μL of Stop Solution and read at 450 nm

Total time: 3 hours

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