Human IL-6 ELISA Kit

Catalog No. EZHIL6

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

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.

The EMD Millipore Human IL-6 ELISA Kit is a Sandwich Enzyme- Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody. This kit is specifically designed for the accurate quantification of human IL-6 from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

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Kit Components

- Human IL-6 Pre-Coated Plate (Part No. CS210043): One 96-well plate,
- Human IL-6 Detection Antibody (Part No. CS210044): One 12 mL bottle,
- Human IL-6 Standard (Part No. CS210032): One lyophilized vial,
- Avidin HRP A (Part No. CS210051): One 12 mL bottle,
- Assay Buffer A (Part No. CS210062): One 25 mL bottle,
- Wash Buffer (20X) (Part No. CS210053): One 50 mL bottle,
- Substrate Solution F (Part No. CS210054): One 12 mL bottle,
- Stop Solution (Part No. CS213422): One 12 mL bottle,
- Plate Sealers (Part No. CS210056): One 4 pack.

Materials Not Supplied

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage & Stability Information

Store kit and unopened components at 2℃ to 8℃

Stability: Kit components are stable for a minimum of 4 months from the date of receipt if stored and handled as described below.

Reagent Precautions

- **Safety Warnings and Precautions:** This kit is designed for research use only and is not recommended for internal use in humans or animals. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.
- This kit is not for use in diagnostic procedures.
- Caustic Material: Stop Solution. The stop solution contains acid which is harmful if swallowed or inhaled; avoid contact with skin and eyes; wash areas of contact immediately with water. **Caution: Eye, hand, face, and clothing protection should be worn when handling this material.**
- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents
- The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and a failure to produce accurate data.

Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantification.

Cell Culture Supernatant
If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70℃. Avoid repeated freeze -thaw cycles.

Serum
Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70℃. Avoid repeated freeze-thaw cycles.

Plasma
Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70℃. Avoid repeated freeze-thaw cycles.

Reagent Preparation

Follow the directions below to prepare reagents and solutions necessary for performing the Human IL-6 ELISA Kit.

1. 20X Wash Buffer: The Wash Buffer provided is supplied as a 20X concentrate. Verify that it does not contain precipitate prior to diluting it to 1X. If necessary, warm to room temperature (20℃ to 25℃) prior to dilution. Dilute the conce ntrate to 1X strength using Milli-Q™ or deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.
2. Human IL-6 Standard: Reconstitute the lyophilized Human IL-6 Standard by adding the volume of Assay Buffer A indicated on the vial label to make the 20 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. In general, samples are analyzed without dilutions. However if dilutions are required, use assay Buffer A as the sample diluent.

Assay Protocol

1. Prepare the reagents as described in the Reagent Preparation section.
2. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Identify the number of reaction wells needed and remove any unneeded strip wells (8 wells per strip) from the plate holder. To do so carefully turn the plate upside down and push the strips from the bottom out through the plate. Re-seal the extra strips in the resealable bag with desiccant and store in the refrigerator for future use.
4. Prepare 500 µL of the 500 pg/mL top standard by diluting 12.5 µL of the standard stock solution in 487.5 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 500 pg/mL top standard in separate tubes using As- say Buffer A as the diluent. Thus, the human IL-6 standard concentrations in the tubes are 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/ mL, 15.6 pg/mL, and 7.8 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).

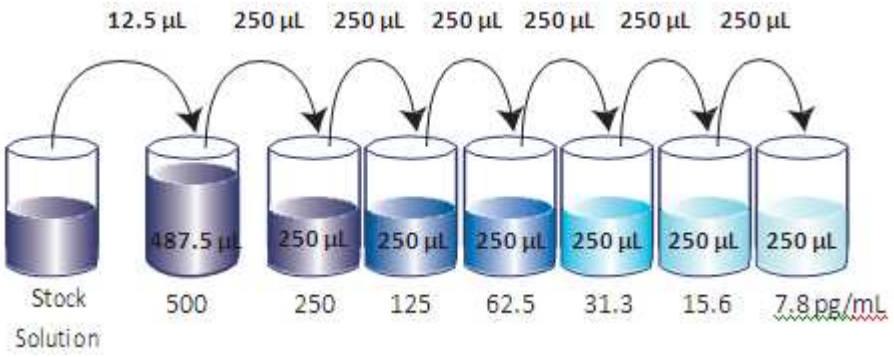
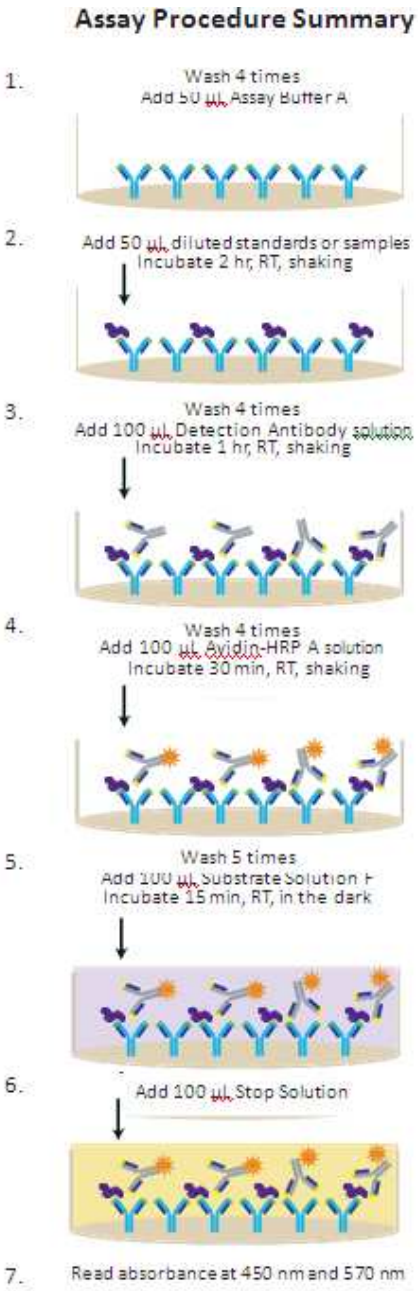


Figure 1: Schematic of serial dilutions and concentrations (ng/mL) for preparation of standards

5. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
6. Add 50 µL of Assay Buffer A to each well that will contain either standard dilutions or samples.
7. Add 50 µL of standard dilutions or samples to the appropriate wells.

8. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
10. Add 100 µL of Human IL-6 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
11. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
12. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
13. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
14. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing human IL-6 should turn blue in color with intensity proportional to its concentration. It is not necessary to seal the plate during this step.
15. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
16. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary



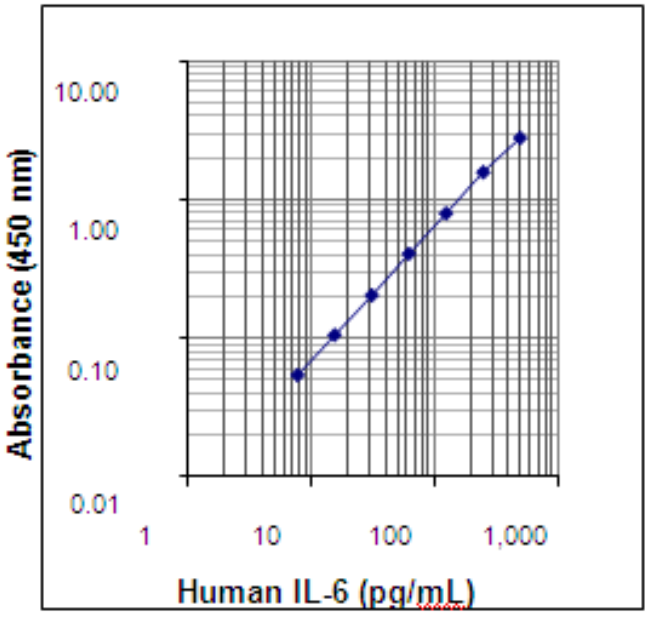
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity

No cross-reactivity was observed when this kit was used to analyze the mouse IL-6 and following recombinant cytokines/chemokines at up to 50 ng/mL.
Human:IL-1a, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-11, IL-12/IL-23 (p40), IL-12(p70), IL-13, Il-15, IL-17A, IL-17A/F, IL-17E, IL-22, IL-23, IL-27, FGF-basic, G-CSF, IFN-g, MCP-1/CCL2, RANTES/CCL, SDF-1a, TWEAK, TGF-b1, TNF-a, TNF-b, VEGF-165

Sensitivity

The minimum detectable concentration of IL-6 is 1.6 pg/mL.

Recovery

Recombinant IL-6 (250, 125 and 62.5 pg/mL) was spiked into 4 human serum samples and then analyzed with Human IL-6 ELISA kit. On average, 96.8 % of the cytokine was recovered from serum samples.

Linearity

Four human serum samples with high concentrations of IL-6 were diluted 1:2, 1:4, 1:8 with Assay Buffer A to produce samples with values within the dynamic range and then assayed. On average, 107 % of the expected cytokine was detected from serum samples.

Intra-Assay Statistics

Sixteen replicates of each of two samples containing different IL-6 concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	241.8	58.7
Standard Deviation	10.3	6.7
% CV	4.3	11.3

Inter-Assay Statistics

Two samples containing different concentrations of IL-6 were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	249.5	64.1
Standard Deviation	11.1	8.3
% CV	4.5	13.0

Biological Samples

Human PBMC (1 x 10^6 cells/mL) were stimulated with 100 ng/mL LPS at 37°C for overnight. Cell culture supernatants were collected and assayed for the levels of natural human IL-6. IL-6 concentration was 75,200 pg/mL in LPS-stimulated samples and undetectable in unstimulated samples.

Troubleshooting Guide

Problem	Probable Cause	Solution
Background is high	Background wells were contaminated	Avoid cross-well contamination by using the sealer appropriately. Use multichannel pipettes without touching the reagents on the plate.
	Matrix used has endogenous analyte	Check the matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No Signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Avidin-HRP was not added.	Add Avidin-HRP according to protocol and continue
	Substrate solution was not added	Add substrate solution and continue
	Wash buffer contains sodium azide	Avoid sodium azide in the Wash Buffer.
Low or poor signal for the standard curve	Standard was incompletely reconstituted or was inappropriately stored	Reconstitute standard according to protocol. Store reconstituted standard in appropriate vials and store at -70℃
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and correct reagent volume.
	Incubations done at inappropriate temperature, timing or agitation	Assay conditions need to be checked

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated O.D.'s	Standard reconstituted with less volume than required	Reconstitute lyophilized standard with correct volume of solution recommended in the protocol.
	Plate incubation was too long	Decrease incubation time
	Detection antibody incubation time is too long	Decrease detection antibody incubation time
	Avidin-HRP incubation time is too long	Decrease Avidin HRP incubation time
	Substrate solution incubation time is too long	Decrease Avidin HRP incubation time
Sample readings are out of range	Sample contain no or below detectable levels of analyte	If samples are below detectable levels it may be possible to use higher sample volume.
	Sample contain analyte concentrations greater than highest standard point	Sample may require dilution and reanalysis
High variation in sample and/or standards	Multichannel pipette errors	Calibrate the pipettes
	Plate washing was not adequate or uniform	Make sure pipette tips are tightly secured. Confirm all reagents are removed completely in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before pipetting.
	Samples may have high particulate matter	Remove the particulate matter by centrifugation.
	Insufficient plate agitation	The plate should be agitated during all incubation steps using an ELISA plate shaker at a speed where solutions in wells are within constant motion without splashing.
	Cross-well contamination	When reusing plate sealers check that no reagent has touched the sealer. Care should be taken when using the same pipette tips used for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

Microtiter Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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