RAPID MAX™

Human IL-6 ELISA Kit

ELISA Kit for Accurate Cytokine Quantitation from Cell Culture Supernatant, Serum, Plasma, and Other Biological Fluids

Cat. No. 430519

BioLegend®

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General Information

Important Information

It is highly recommended to read this manual in its entirety before using this product. Do not use this kit beyond the expiration date.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.

Warnings

This manual highlight particularly important remarks as follows:

WARNING	Indicates a risk of death or serious injury, or damage to property.
CAUTION	Indicates a risk of moderate or minor injury, or damage to property.
IMPORTANT	Indicates critical information that must be observed.

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, such as rheumatoid arthritis.

Principle of the Assay

The BioLegend RAPID MAX™ Human IL-6 ELISA Kit is a one step, one wash, 80-minute enzyme-linked immunosorbent sandwich assay (ELISA) with a pre-coated, 96-well strip plate that immobilizes the biotinylated capture antibody. Standards and samples are added into the wells, followed by an antibody cocktail. The antibody cocktail consists of a biotinylated rat monoclonal capture antibody and an HRP-conjugated rat monoclonal detection antibody. This kit is specifically designed for the accurate quantitation of human IL-6 from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Materials Provided

Description	Quantity	Volume (per bottle)	Part No.
RAPID MAX™ Pre-coated 96-well Strip Microplate	1 plate		750001894
Human IL-6 Detection Antibody Concentrate (200X)	1 vial	60 µL	750001891
Human IL-6 Capture Antibody Concentrate (200X)	1 vial	60 µL	750001887
Human IL-6 Standard	1 vial	lyophilized	750001954
Assay Diluent A (5X)	1 bottle	12 mL	750002142
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	1 pack		78101

Materials to be Provided by the User

- 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
- Phosphate-Buffered Saline (PBS): 8.0 g NaCl, 1.16 g Na2HPO4, 0.2 g KH2PO4, 0.2 g KCl, add deionized water to 1.0 L, pH to 7.4, 0.2 µm filtered.

Storage Information

Store unopened kit components between 2-8 °C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2-8 °C for up to one month.

Opened or Reconstituted Components	
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at ≤ -70 °C for up to one month. Avoid repeated freeze-thaw cycles.
Detection Antibody Concentrate (200X)	Store opened reagents between 2-8 °C and use within one month.
Capture Antibody Concentrate (200X)	
Assay Diluent A (5X)	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings

WARNING Observe the following precautions prior to use.

- Reagents that contain preservatives may be harmful if ingested, inhaled, or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details: biolegend.com/msds
- Substrate Solution F is harmful if inhaled or ingested. Avoid contact with skin, eyes, and clothing.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
- Stop Solution contains strong acid. Wear eye, hand, and face protection.
- Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling

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

Cell Culture Supernatant

If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at ≤ -70 °C. 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 °C. 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 °C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation

IMPORTANT Dilute all reagents immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 L of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring it to room temperature and vortex until dissolved.
2. Dilute the 5X Assay Diluent A to 1X using Phosphate-Buffered Solution (PBS). For example, make 10 mL of 1X Assay Diluent A by adding 2 mL of 5X Assay Diluent A to 8 mL PBS.
3. Reconstitute the lyophilized Human IL-6 Standard by adding the volume of Assay Diluent A indicated on the kit CoA to make 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.
4. Prepare the antibody cocktail by adding 60 µL of Human IL-6 Detection Antibody Concentrate (200X) and 60 µL of Human IL-6 Capture Antibody Concentrate (200X) in 11.88 mL Assay Diluent A. **Note:** Only prepare the entire stock of antibody cocktail if running a full plate. If only part of the plate is being run, prepare only the necessary amount of antibody cocktail. Do not store

additional antibody cocktail. The capture and detection antibody concentrates must be stored at 200X for long term stability.

5. In general, serum and plasma samples are analyzed with a 2-fold dilution and cell culture supernatants are analyzed with a 10-fold dilution. Use Assay Diluent A as the sample diluent.

Technical Hints

- Follow all instructions outlined in the Assay Procedure section with particular attention to incubation times.
- Avoid contamination between wells by changing pipette tips between the addition of each standard and between each addition of the antibody cocktail. Use separate reservoirs for each reagent. Pipette against the well while avoiding dipping the pipette tip into the sample for best results.
- Reduce time between loading reagents by ensuring that addition is uninterrupted. Use of a multichannel pipette can also decrease differences in loading time between wells.
- This plate does not require shaking during the incubation step. Incubate on bench top without shaking.
- Load Substrate Solution and Stop Solution in the same order on the plate.
- Ensure that all liquid is removed from the wells after washing by tapping them firmly on clean absorbent paper. Tap out liquid after every soaking step.

Assay Procedure

IMPORTANT	Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit. To ensure correct incubation, use a multichannel pipette to load the antibody cocktail.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate and loaded using a multichannel pipette. A standard curve is required for each assay.
2. If all the microplate strips will not be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 500 µL of the 1000 pg/mL top standard by diluting 25 µL of the standard stock solution in 475 µL of Assay Diluent A. Perform six two-fold serial dilutions of the 1000 pg/mL top standard in separate tubes using Assay Diluent A as the diluent. Thus, the human IL-6 standard concentrations in the tubes are 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, and 15.6 pg/mL, respectively. Assay Diluent A serves as the zero standard (0 pg/mL).

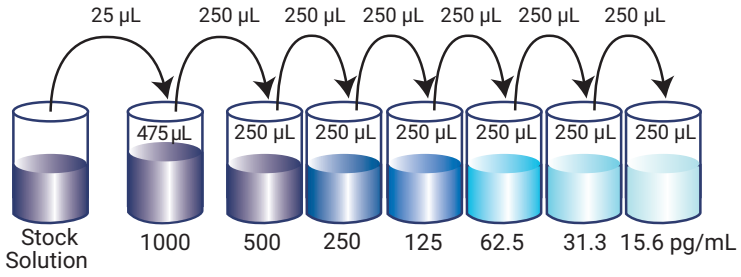

Figure 1. Illustration of dilution procedure.

4. Add 50 µL of standard dilutions or samples per wells.
5. Add 50 µL of the antibody cocktail to each well.
6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for one hour on benchtop with no shaking.
7. Discard the contents of the plate into a sink, then wash the plate five times by adding 300 µL 1X Wash Buffer per well. Soak for 30 seconds to one minute per wash. Blot any residual buffer by firmly tapping the plate upside down on absorbent paper after each soak.
8. Add 100 µL of Substrate Solution F to each well and incubate the plate at room temperature for 20 minutes in the dark. Wells containing human IL-6 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step. **Note:** Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.
9. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
10. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 or 620 nm, the absorbance at 570 or 620 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

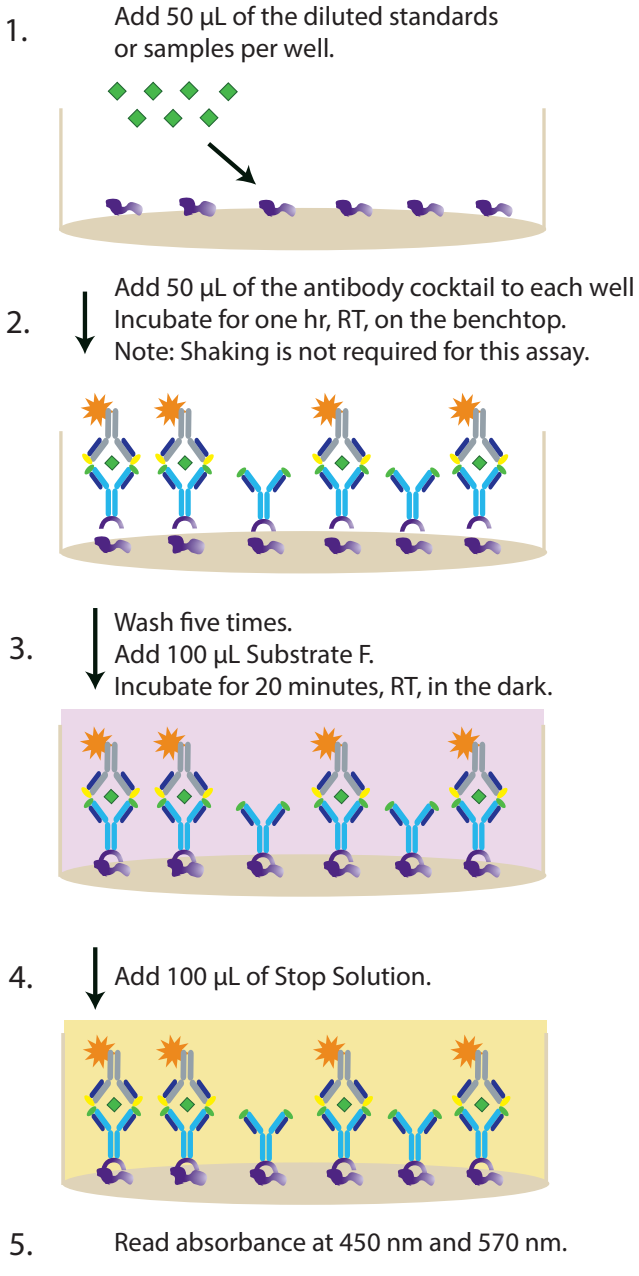

Figure 2. Illustration of assay procedure.

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 reanalyzed at a higher (or lower) dilution as appropriate.

Typical Data

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

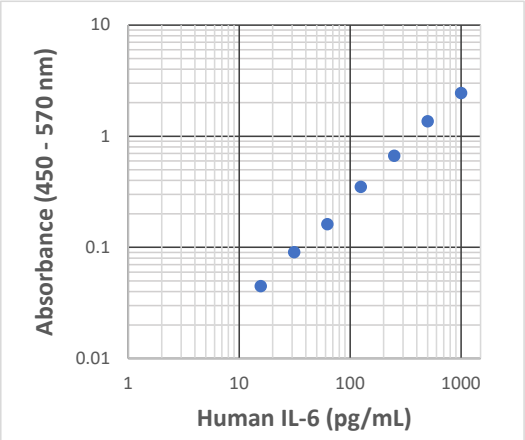

Figure 3. Graph of standard curve for demonstration purposes.

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-1α, IL-1β, 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-γ, MCP-1/CCL2, RANTES/CCL, SDF-1α, TWEAK, TGF-β1, TNF-α, TNF-β, VEGF-165

Sensitivity

The minimum detectable concentration (minDC) of human IL-6 is 5.99 pg/mL. The minDC was determined by adding two standard deviations to the mean O.D. of 24 zero standard replicates and calculating the corresponding concentration.

Recovery

Recombinant or natural IL-6 at three different concentrations was spiked into human samples of serum, EDTA plasma, citrate plasma, and heparin plasma, and then analyzed with the RAPID MAX™ Human IL-6 ELISA kit.

Sample Type	% Recovery, Average
Serum (n=4)	95.2
EDTA Plasma (n=4)	100.0
Citrate Plasma (n=4)	109.4
Heparin Plasma (n=4)	93.7
Cell Culture Supernatant (n=2)	96.5

Linearity

Human samples were first diluted to the recommended dilution factor, spiked with high concentrations of IL-6, then diluted 2-fold in serial with Assay Diluent A to produce samples with values within the dynamic range and assayed.

Sample Type	% Linearity, Average
Serum (n=5)	95.9
EDTA Plasma (n=4)	100.7
Citrate Plasma (n=5)	99.1
Heparin Plasma (n=5)	94.7
Cell Culture Supernatant (n=3)	103.8

Intra-Assay Statistics

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

Concentration	Sample 1	Sample 2
Number of Replicates	14	14
Mean Concentration (pg/mL)	33.9	98.5
Standard Deviation	2.9	8.9
% CV	8.6	9.0

Inter-Assay Statistics

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

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	32.3	111.8
Standard Deviation	4.7	13.1
% CV	14.6	11.7

Biological Samples

Human PBMC (1 x 10^6 cells/mL) were stimulated with 1 ug/mL LPS at 37 °C for 3 days. Cell culture supernatants were collected and assayed for the levels of natural human IL-6. IL-6 concentration was 18,560 pg/mL in LPS-stimulated samples and undetectable in unstimulated samples.

Troubleshooting

Problem	Probable Cause	Solution
High background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents. Do not agitate plate.
	Insufficient washes	Increase soaking time between washes prior to addition of substrate solution.
	Substrate Solution was contaminated	Substrate Solution should be clear and colourless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Capture Antibody, samples/ standard, or Substrate Solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing, or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at ≤ -70 °C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Check calculation for reconstitution. If further dilution is required, dilute to the correct concentration and rerun the assay.
	Standards/samples, antibody cocktail, or substrate solution were incubated for too long	Rerun the assay and follow the protocol.

Problem	Probable Cause	Solution
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than the highest standard point	Samples may require dilution and reanalysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipettes are calibrated accurately.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not use plate sealers. Always change pipette tips between reagent additions. Ensure that pipette tips do not touch the reagents in the wells.

ELISA Plate Template

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

Figure 4. ELISA Plate Template

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RAPID MAX™ Kits are manufactured by BioLegend Inc.
8999 BioLegend Way
San Diego, CA 92121
Tel: 1.858.768.5800
Tel US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)
Fax: 1.877.455.9587
Email: info@biolegend.com
biolegend.com

For a complete list of world-wide BioLegend offices and distributors, please visit our website at: biolegend.com

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