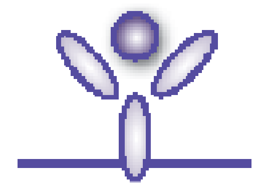
LEGEND MAX™

ELISA Kit



Human IL-34

Cat. No. 439607

ELISA Kit for Accurate Quantitation of Human IL-34 from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read 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.

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Introduction:

IL-34 is a secreted glycoprotein, synthesized as a 242 amino acid precursor that contains a 20 amino acid signal sequence and a 222 amino acid mature chain. Mature human IL-34 forms a homodimer, consisting of 39 kD monomers. IL-34 is highly conserved among mammals. Human IL-34 has sequence identity of 99.6%, 72% and 71% with chimpanzee, rat, and mouse IL-34, respectively.

IL-34 is widely expressed in many tissues, but most abundant in the spleen. IL-1β and TNF-α can induce the expression of IL-34 mRNA in osteoblasts. Also, IL-34 is induced in macrophages infected with equine infectious anemia virus (EIAV). Functionally, IL-34 increases monocyte viability, induces macrophage proliferation, and synergizes with other cytokines to generate macrophages and osteoclasts from cultured progenitors. Although IL-34 has no sequence homology with M-CSF (also CSF-1), it binds to the CSFR, the same receptor for M-CSF. IL-34 and M-CSF show an equivalent ability to support cell growth or survival, but they are not identical in biological activity and signal activation. These cytokines also have differing abilities to induce the production of chemokines (MCP-1 and eotaxin-2) in primary macrophages.

IL-34 has been implicated in some disease processes. For example, IL-34 expression is associated with synovitis severity in rheumatoid arthritis (RA) patients. IL-34 elevation in plasma from RA patients was decreased after the administration of disease-modifying anti-rheumatic drugs (DMARDs). Also, strategies of targeting CSF1/CSF1R are already in preclinical and clinical studies for treatment of inflammatory diseases. In addition, it has been shown that IL-34 can enhance the neuroprotective property of microglia and therefore, this may be an effective approach against oAβ (oligomeric β-amyloid) neurotoxicity in Alzheimer's disease.

The BioLegend LEGEND MAX™ Human IL-34 ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal mouse anti-human IL-34 capture antibody. The detection antibody is a biotinylated monoclonal mouse anti-human IL-34 antibody. This kit specifically quantitates human IL-34 from cell culture supernatant, serum, plasma (EDTA) and other biological fluids. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-Human IL-34 Pre-coated 96-well Strip Microplate	1 plate		79861
Human IL-34 Detection Antibody	1 bottle	12 mL	79862
Human IL-34 Standard	1 vial	lyophilized	79872
Matrix A	1 bottle	lyophilized	78303
Avidin-HRP D	1 bottle	12 mL	78237
Assay Buffer B	1 bottle	25 mL	79128
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	4 sheets		78101

Materials to be Provided by the End-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

Storage Information:

Store unopened kit components between 2°C and 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°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution and Matrix A can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Matrix A	
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP D	
Assay Buffer B	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. 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 (www.biolegend.com/MSDS).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. 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, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell and Tissue 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 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.

*Note: Citrate and Heparin plasma samples are not recommended.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with 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. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.

2. Reconstitute the lyophilized Matrix A by dispensing 2 mL of deionized water into the vial. Allow the reconstituted Matrix A to sit at room temperature for 15 minutes, vortex to mix completely.

3. Prepare 2 mL of diluted Matrix A reconstituted as above based on sample types: a) **For EDTA plasma samples and cell culture supernatant samples containing 10% fetal bovine serum**, reconstituted Matrix A should be diluted 10X with Assay Buffer B. For example, dilute 200 µL of Matrix A with 1.8 mL of Assay buffer B. b) **For serum samples,** reconstituted Matrix A should be diluted 20X with Assay Buffer B. For example, dilute 100 µL of Matrix A with 1.9 mL of Assay Buffer B.

   Note: Only 2 mL of diluted Matrix A is needed for each standard curve.

4. Reconstitute the lyophilized Human IL-34 Standard by adding the volume of Assay Buffer B to make the 100 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.

5. In general, no sample dilutions are required. If dilutions are necessary, samples should be diluted with Matrix A prepared above. Matrix A concentrations to use should be based on sample types as specified above in 3.

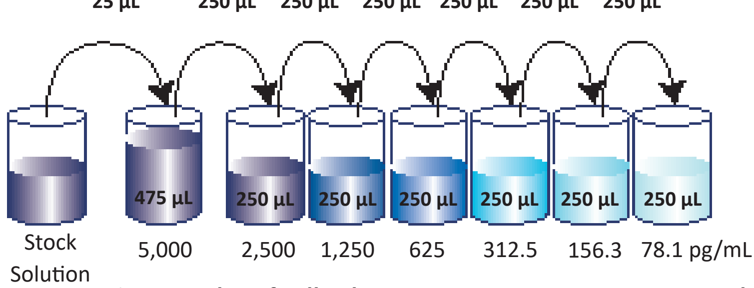
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

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. A standard curve is required for each assay.

2. If not all microplate strips will 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 5,000 pg/mL top standard by diluting 25 µL of the standard stock solution in 475 µL of Assay Buffer B. Perform six two-fold serial dilutions of the 5,000 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the Human IL-34 standard concentrations in the tubes are 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312.5 pg/mL, 156.3 pg/mL, and 78.1 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).



4. **For measuring samples of cell culture supernatant, serum or EDTA plasma samples:**
   a) Add 50 µL of diluted Matrix A (see **Reagent and Sample Preparation, 3)** to each well that will contain standard dilutions. Add 50 µL of Assay Buffer B to each well that will contain samples.
   b) Add 50 µL of standard dilutions to the wells for standards. Add 50 µL of samples to the wells for samples.

5. Seal the plate with the plate sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.

6. Discard the contents of the plate into a sink. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.

7. Add 100 µL of the Human IL-34 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 6.

9. Add 100 µL of Avidin-HRP D solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

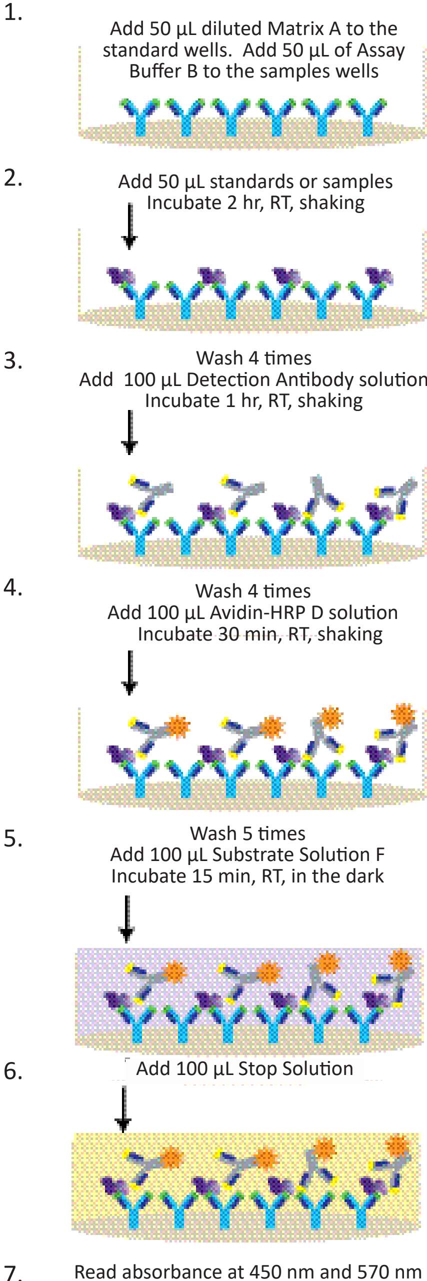
10. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 6. For this final wash, soak wells in 1X Wash Buffer for 1 minute for each wash. This will help minimize background.

11. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells with human IL-34 should turn a blue color, with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

12. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

13. 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



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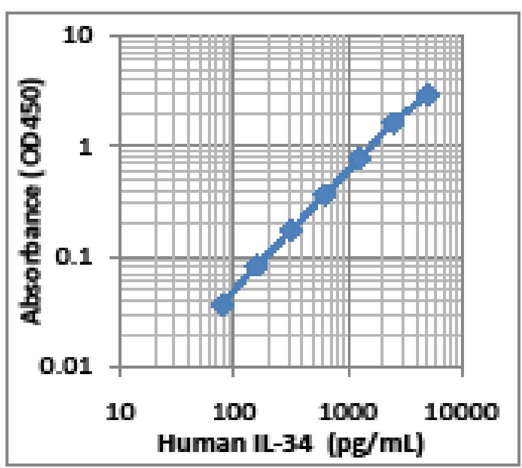
Calculation of Results:

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 at BioLegend 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 following human recombinant proteins, each at a concentration of 50 ng/mL:

IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, IL-22, IL-23, IL-32α, IL-33, TNF-α, TNF-β, EGF, G-CSF, M-CSF, GM-CSF, SDF-1α,

PDGF-BB, GROα, CCL20, CCL22, MCP-1, MCP-3, MIP-1α, CX3CL1, Eotaxin, TGF-α, IP-10, IFN-α.

Sensitivity: The minimum detectable concentration is 23.0 ± 5.2 pg/mL (n=6).

Recovery: Three levels of recombinant Human IL-34 (1,250 pg/mL, 625 pg/mL, 312.5 pg/mL) were spiked into each of 7 human serum samples, and then analyzed with the LEGEND MAX™ Human IL-34 ELISA kit. On average, 115.6 % of the protein was recovered from the serum samples.

Linearity: Six human serum samples spiked with high concentrations of human IL-34 were diluted with the appropriate matrix to produce samples with values within the dynamic range and then assayed. Linearity of dilution averaged 93.3%.

Intra-Assay Precision: Two samples with different IL-34 concentrations were tested on one plate with 12 replicates.

Concentration	Sample 1	Sample 2
Number of Replicates	12	12
Mean Concentration (pg/mL)	586.6	2,425.9
Standard Deviation	39.2	127.1
% CV	6.7	5.2

Inter-Assay Precision: Two samples containing different concentrations of IL-34 were tested in five independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	5	5
Mean Concentration (pg/mL)	363.3	2078.8
Standard Deviation	19.7	113.7
% CV	5.4	5.5

Biological Samples:

Serum and Plasma: Normal human serum (n=28) and EDTA plasma (n=11) samples were assayed for basal levels of human IL-34. Sample IL-34 levels range from non-detectable to 5,000 pg/mL, with a mean of 404 pg/mL and 67% sample detectability.

Cell Culture Supernatant: Human U937 cells were differentiated for two days after stimulation with 10 nM PMA and cells were washed and restimulated with LPS (100 ng/mL) alone, LPS plus IFN-γ (20 ng/mL), or LPS plus IFN-γ and TNF-α (20 ng/mL). Cell culture supernatants were removed after 24 hours and assayed for human IL-34. Human IL-34 concentrations measured 38 pg/mL, 162 pg/mL, and 180 pg/mL in LPS, LPS plus IFN-γ, and LPS plus IFN-γ and TNF-α samples, respectively. No IL-34 was detected in unstimulated control samples.

Troubleshooting Guide:

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.
	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 or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	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.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
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 highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	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 reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

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

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