



LEGEND MAX[™]
ELISA Kit



Human IL-17A/F

Cat. No. 435807

ELISA Kit for Accurate Quantitation of Human IL-17A/F
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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LEGEND MAX™ Human IL-17A/F ELISA Kit

Introduction:

Human IL-17A/F is a heterodimeric member of the IL-17 family. It is a pro-inflammatory cytokine secreted by activated Th17 cells in response to bacteria and fungi. There is great interest in the members of the IL-17 family for their role, not only in adaptive immunity and inflammation, but also in a variety of auto-immune diseases, such as Crohn's disease, ulcerative colitis, psoriasis, multiple sclerosis, rheumatoid arthritis, asthma, and lupus.

IL-17A/F consists of the monomeric units of IL-17A and IL-17F, which share 50% amino acid sequence identity. It has been shown to be a naturally expressed and biologically active molecule. While much has been studied about IL-17A and IL-17F, it is still unclear how each distinctively induces the inflammatory response, and what role IL-17A/F plays in it. It is known that all three cytokines are produced in different amounts, and each has a different degree of pro-inflammatory effects. All seem to interact with the IL-17RA/IL-17RC receptor complex, and signal via the NF- κ B pathway to induce secretion of inflammatory cytokines and recruit granulocytes.

BioLegend's LEGEND MAX™ Human IL-17A/F ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a polyclonal goat anti-human IL-17A/F capture antibody. The detection antibody is a biotinylated monoclonal mouse anti-human IL-17A/F antibody. This kit is specifically designed for the accurate quantitation of human IL-17A/F from cell culture supernatant, serum, plasma and other biological fluids. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-Human IL-17A/F Pre-coated 96-well Strip Microplate	1 plate		79680
Human IL-17A/F Detection Antibody	1 bottle	12 mL	79681
Human IL-17A/F Standard	1 vial	lyophilized	79683
Avidin-HRP D	1 bottle	12 mL	78237
Matrix C (for serum and plasma samples only)	1 vial	lyophilized	78316
Assay Buffer A	1 bottle	25 mL	78232
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

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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 C can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Matrix C	
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP D	
Assay Buffer A	
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 Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at $< -70^{\circ}\text{C}$. 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 $1,000 \times g$. Remove serum layer and assay immediately or store serum samples at $< -70^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at $1,000 \times g$ within 30 minutes of collection. Assay immediately or store plasma samples at $< -70^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

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

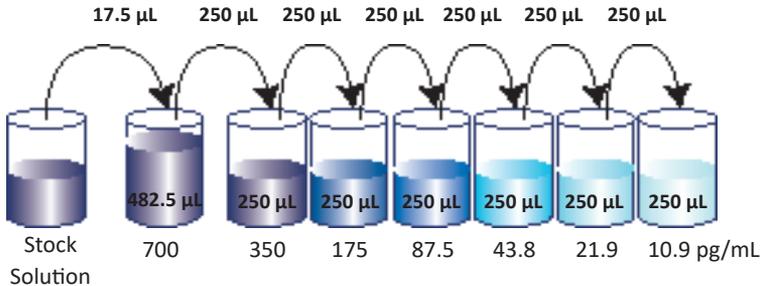
1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. If serum or plasma samples will be assayed, reconstitute the lyophilized Matrix C by dispensing 4 mL of deionized water into the vial. Allow the reconstituted Matrix C to sit at room temperature for 15 minutes, vortex to mix completely.
3. Reconstitute the lyophilized Human IL-17A/F Standard by adding the volume of Assay Buffer A to make the 20 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.
4. In general, samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer A for diluting cell culture supernatant samples and Matrix C for diluting plasma and serum samples.

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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 700 pg/mL top standard by diluting 17.5 μL of the standard stock solution in 482.5 μL of Assay Buffer A. Perform six two-fold serial dilutions of the 700 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human IL-17A/F standard concentrations in the tubes are 700 pg/mL , 350 pg/mL , 175 pg/mL , 87.5 pg/mL , 43.8 pg/mL , 21.9 pg/mL and 10.9 pg/mL , respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. 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.
5. **For measuring samples of cell culture supernatant:**
 - a) Add 50 μL of Assay Buffer A to each well that will contain either standard dilutions or samples.
 - b) Add 50 μL of standard dilutions or samples to the appropriate wells.

For measuring serum or plasma samples:

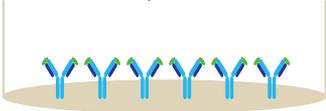
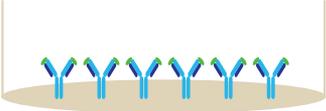
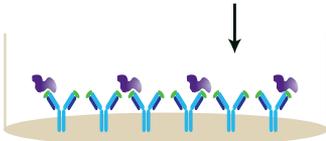
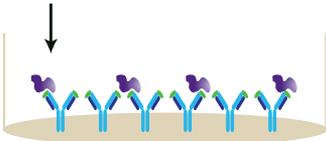
- a) Add 50 μL of Matrix C to each well that will contain the standard dilutions. Add 50 μL of Assay Buffer A to each well that will contain

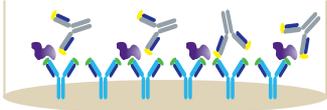
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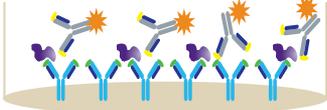
samples.

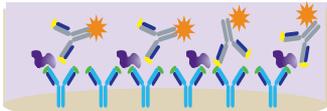
- b) Add 50 μ L of standard dilutions to the wells containing Matrix C. Add 50 μ L of diluted serum or plasma samples to the wells containing Assay Buffer A.
6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
8. Add 100 μ L of Human IL-17A/F Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
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 μ L of Avidin-HRP D solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
11. 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.
12. Add 100 μ L of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing human IL-17A/F should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
13. Stop the reaction by adding 100 μ L of Stop Solution to each well. The solution color should change from blue to yellow.
14. 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.

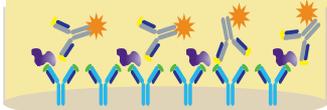
Assay Procedure Summary

- | | |
|--|--|
| <p>1. For measuring cell culture supernatant:</p> <p>Wash 4 times
Add 50 µL Assay Buffer A to standard and sample wells</p>  | <p>For measuring serum or plasma:</p> <p>Wash 4 times
Add 50 µL Matrix C to standard wells
Add 50 µL Assay Buffer A to sample wells</p>  |
|--|--|
- | | |
|---|---|
| <p>2. Add 50 µL diluted standards to standard wells
Add 50 µL samples to sample wells
Incubate 2 hr, RT, shaking</p>  | <p>Add 50 µL diluted standards to standard wells
Add 50 µL of samples to sample wells
Incubate 2 hr, RT, shaking</p>  |
|---|---|
- 3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking**


- 4. Wash 4 times
Add 100 µL Avidin-HRP D solution
Incubate 30 min, RT, shaking**


- 5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 15 min, RT, in the dark**


- 6. Add 100 µL Stop Solution**


- 7. Read absorbance at 450 nm and 570 nm**

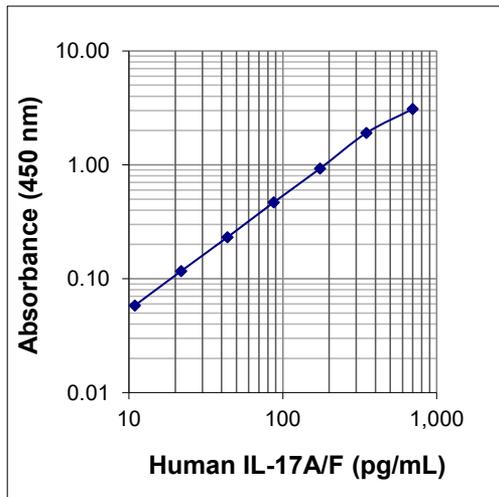
Calculation of Results:

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: There was negligible cross-reactivity (<0.1%) with human IL-17A. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines/chemokines, each at up to 50 ng/mL.

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Human	IL-17E, IL-17F, IL-1 α , IL-1 β , IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p40, IL-12 p70, IL-13, IL-16, IL-18, IL-22, IL-23, IL-32 α , IL-33, IFN- α , IFN- β , IFN- γ , IFN λ 2, IFN- ω , TNF- α , TNF- β , RANTES, TGF- α , EGF, ECAM-1, FGF- β
Mouse	IL-17A, IL-17E, IL-17F, IL-17A/F

Sensitivity: The minimum detectable concentration is approximately 2.58 pg/mL (average of 2.19 ± 0.40 pg/mL in 5 assays).

Recovery: Recombinant human IL-17A/F, at concentrations of 363.5, 90.9, and 22.8 pg/mL, was spiked into 10 human serum samples and then analyzed with the LEGEND MAX™ Human IL-17A/F ELISA Kit. On average, 98.4% of the protein was recovered from the serum samples.

Linearity: Recombinant human IL-17A/F spiked into four human serum samples was diluted with Matrix C to produce samples with values within the dynamic range and then assayed with the kit to determine the dilution linearity. The linearity of dilution ranged from 76 to 119%. On average, 89.7% linearity of dilution was observed.

Intra-Assay Precision: Two samples with different human IL-17A/F concentrations were tested with 12 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	12	12
Mean Concentration (pg/mL)	351.4	23.1
Standard Deviation	6.0	1.3
% CV	1.7	5.6

Inter-Assay Precision: Two samples with different concentrations of human IL-17A/F were assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	337.9	19.6
Standard Deviation	23.2	1.3
% CV	6.9	6.4

Biological Samples:

Serum/PLasma - Twenty normal human serum samples and 18 normal human plasma samples were assayed for basal levels of human IL-17A/F. No samples had detectable concentrations.

Cell Culture Supernatant - Human PBMCs were isolated and plated in a 12-well plate at $\sim 2 \times 10^6$ cells/mL in RPMI. Cells were stimulated with CD3 + CD28 (2 + 2 μ g/mL), PMA + Ionomycin (20 + 500 ng/mL), or LPS (100 ng/mL), and supernatants were removed at various days and assayed for human IL-17A/F. There was a time-dependent increase in human IL-17A/F from all stimulated cells, with CD3 + CD28 stimulation producing the highest levels at

1,100 pg/mL by day 3, and LPS stimulation producing the lowest levels at 32 pg/mL.

Troubleshooting Guide:

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.

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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 (NaN ₃)	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.

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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