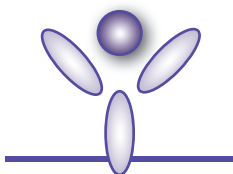




LEGEND MAX[™]

ELISA Kit



Mouse IL-25 (IL-17E)

Cat. No. 447107

ELISA Kit for Accurate Quantitation of Mouse
IL-25 (IL-17E) 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™ Mouse IL-25 (IL-17E) ELISA Kit

Introduction:

IL-25, also known as IL-17E, is a pro-inflammatory cytokine encoded by the *IL25* gene located on chromosome 14. The IL-17 family consists of IL-17A, IL-17B, IL-17C, IL-17F, IL-17A/F, and IL-17E. The IL-17 family has a similar protein structure with four conserved cysteine residues and two disulfide bonds. IL-25 expression is widely distributed and can be produced by a variety of cells and tissues. IL-25 induces T helper 2 (Th2) expansion and production of cytokines IL-4, IL-5, and IL-13. This is important in eosinophil expansion, regulating gut immunity, and in the response to helminth parasites.

IL-25 has been demonstrated to play a role in allergy and asthma. IL-25 levels are positively correlated with the severity of allergenic rhinitis in patients. Blocking IL-25 has been proposed as an allergy treatment in combination with other therapies. IL-25 has also been associated with the pathogenesis of rheumatoid arthritis and is frequently upregulated in the serum and synovial fluid of patients and in mouse models of rheumatoid arthritis.

BioLegend's LEGEND MAX™ Mouse IL-25 (IL-17E) ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate. The plate is pre-coated with a monoclonal rat capture antibody. The detection antibody is a biotinylated rat monoclonal antibody. This kit is specifically designed for the accurate quantitation of mouse IL-25 from cell culture supernatant, serum, plasma and other biological fluids. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume	Part #
Mouse IL-25 (IL-17E) Pre-Coated 96-well Strip Microplate	1 plate		750000604
Mouse IL-25 (IL-17E) Detection Antibody (200X)	1 bottle	70 µL	750000605
Mouse IL-25 (IL-17E) Standard	1 vial	lyophilized	750000609
Avidin-HRP	1 bottle	12 mL	77897
Assay Diluent D	1 bottle	25 mL	76384
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

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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
- Polypropylene 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 all 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. Store between 2°C and 8°C for up to one month.
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 (200X)*	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP	
Assay Diluent D	
Assay Buffer B	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

*For long term storage the Detection Antibody must be kept at 200X

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).

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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. Reconstitute the lyophilized Mouse IL-25 Standard by adding the volume of Assay Diluent D to make the 16,000 pg/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 15-20 minutes, then briefly vortex to mix completely.
3. In general, it is recommended for serum and plasma samples are run neat, but samples can be diluted with Assay Diluent D to fit within the range of the assay as determined by the end user.

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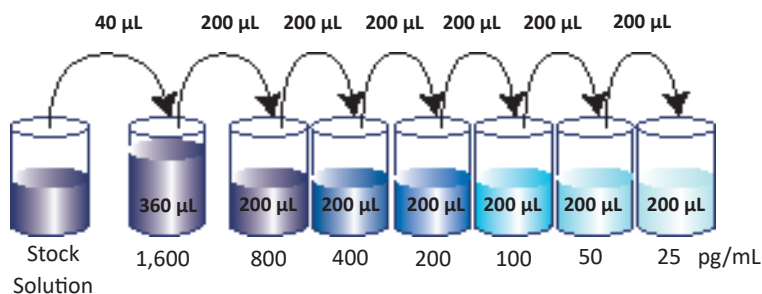
4. Dilute the 200X Detection Antibody in Assay Buffer B immediately prior to use. If the entire plate is to be run, add 60 μL of Detection Antibody to 11.94 mL of Assay Buffer B.

NOTE: Do not dilute the entire detection antibody stock if only part of the plate is to be run. The detection antibody must be stored at 200X for long term stability. The detection antibody must be diluted in Assay Buffer B and the sample in Assay Diluent D for the kit to work accurately.

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 all 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 400 μL of the 1,600 pg/mL top standard by adding 40 μL of the 16,000 pg/mL standard stock solution in 360 μL Assay Diluent D. Perform six two-fold serial dilutions of the 1,600 pg/mL top standard in separate tubes using Assay Diluent D as the diluent. Thus, the Mouse IL-25 standard concentrations in the tubes are 1,600 pg/mL, 800 pg/mL, 400 pg/mL, 200 pg/mL, 100 pg/mL, 50 pg/mL and 25 pg/mL, respectively. Assay Diluent D serves as the zero standard (0 pg/mL).



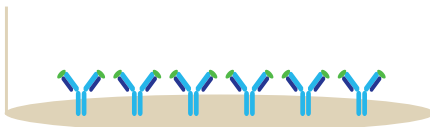
4. Wash the plate 4 times with at least 300 μL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50 μL of Assay Diluent D to each well that will contain either standard dilutions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

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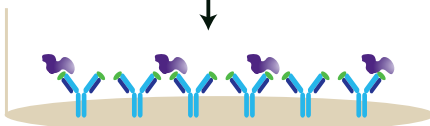
6. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.
7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
8. Add 100 μ L of 1X diluted Mouse IL-25 Detection Antibody solution 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 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 mouse IL-25 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

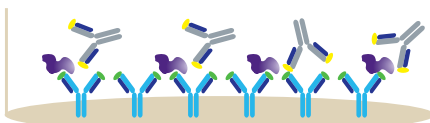
1. Wash 4 times.
Add 50 μ L Assay Diluent D to standard wells and sample wells



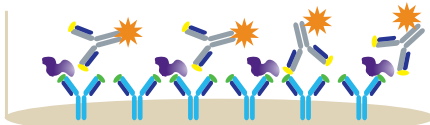
2. Add 50 μ L of standard or sample, incubate 2 hrs., RT, shaking



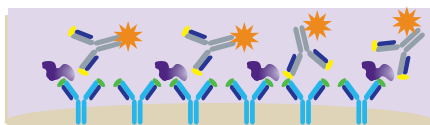
3. Wash 4 times
Add 100 μ L Assay Buffer B diluted Detection Antibody solution
Incubate 1 hr., RT, shaking



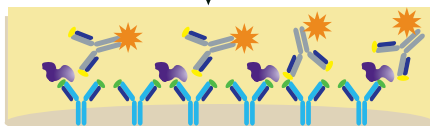
4. Wash 4 times
Add 100 μ L Avidin-HRP solution
Incubate 30 min., RT, shaking



5. Wash 5 times
Add 100 μ L Substrate Solution F
Incubate 15 min., RT, in the dark



6. Add 100 μ L Stop Solution



7. Read absorbance at 450 nm and 570 nm

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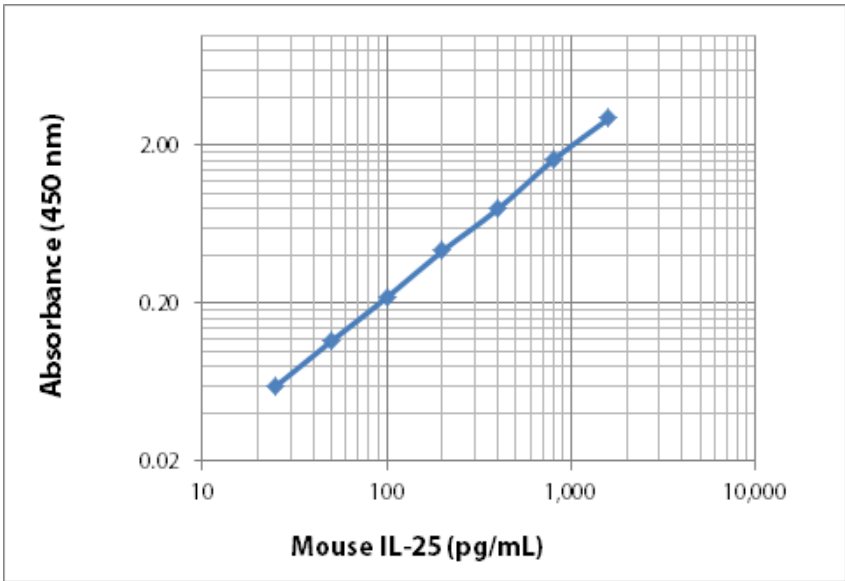
Calculation of Results:

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.



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Performance Characteristics:

Specificity: No cross reactivity was observed when this kit was used to analyze the following recombinant proteins at 50 ng/mL.

Human	IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-12 (p70), IL-13, IL-15, IL-17F, IL-17A/F, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-25, IL-27, IL-33, LT- α , M-CSF, NGAL, Paxillin, SCR, TGF- α
Mouse	IFN- γ , IL-4, IL-5, IL-6, IL-7, IL-10, IL-11, IL-12, IL-13, IL-15, IL-17BR, IL-17F, IL-17A/F, IL-17A, IL-33, IL-34, Isthmin1, M-CSF, MMP-9
Rat	IL-17A, IL-17B

No detection in rat, equine, or rhesus macaque serum.

Sensitivity: The minimum detectable concentration of mouse IL-25 is 2.681 ± 0.459 pg/mL (n=10).

Recovery: Recombinant mouse IL-25 (800, 200, and 50 pg/mL) was spiked into four different mouse strains of pooled serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma with two different lots for each sample. Samples were then analyzed with the LEGEND MAX™ Mouse IL-25 ELISA Kit. No significant difference was observed between different mouse strains.

Sample Type	N	% Recovery
Serum	8	112.5
Citrate Plasma	8	113.3
EDTA Plasma	8	111.8
Heparin Plasma	8	107.5

Linearity: Recombinant mouse IL-25 (1000 pg/mL) was spiked into 4 different mouse strains of pooled serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma with two different lots for each sample. Samples were then diluted 2-fold produce samples within the dynamic range of the kit. Samples were then assayed to determine the dilutional linearity. No significant difference was observed between different mouse strains.

Sample Type	N	% Linearity
Serum	8	88.4
Citrate Plasma	8	83.0
EDTA Plasma	8	83.7
Heparin Plasma	8	83.4

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Intra-Assay Precision: Two samples containing different concentrations of mouse IL-25 were tested on one plate with 15 replicates.

Concentration	Sample 1	Sample 2
Number of Replicates	15	15
Mean Concentration (pg/mL)	467.8	76.9
Standard Deviation	28.6	5.2
%CV	6.1	6.8

Inter-Assay Precision: Two samples containing different concentrations of mouse IL-25 were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	567	111
Standard Deviation	77.4	16.3
%CV	13.6	14.7

Troubleshooting Guide:

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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.

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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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