BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Human IL-31

Cat. No. 445707 1 Plate

445708 5 Plates

ELISA Kit for Accurate Quantitation of Human IL-31 from Cell Culture Supernatant, Serum, and Plasma

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.

Table of Contents

Introduction:

Interleukin 31 (IL-31) is a newly identified member of the gp130/IL-6 cytokine family.It is composed of 144 amino acid and has molecular mass of 16 kD. IL-31 is produced mainly by type 2 T helper cells. The mature form of IL-31 contains a four helix structure. Human IL-31 shares 31% amino acid sequence identity with mouse IL-31.

IL-31 is a pleiotropic cytokine which is associated with immune response, hematopoiesis, and cytokine induction. IL-31 signals through a heterodimer receptor which consists of unique gp130-like receptor chain IL-31RA, and the receptor subunit OSMRβ that is shared with another family member oncostatin M (OSM). When IL-31 binds to the receptor, the complex is able to activate different signaling pathways including JAK/STAT, PI3K/AKT, and MAPK.

Studies on a mouse model show that high levels of IL-31 is associated with developing severe atopic dermatitis. IL-31 receptor expression is also increased in diseased tissues derived from an animal model of airway hypersensitivity. In addition, this cytokine plays a key role in the pathogenesis of inflammatory bowel disease.

The BioLegend LEGEND MAX™ Human IL-31 Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit includes a 96-well strip plate that is pre-coated with a goat polyclonal anti-human IL-31 capture antibody. The detection antibody is a biotinylated goat polyclonal anti-human IL-31 antibody. This kit is specifically designed for the accurate quantitation of human IL-31 from cell culture supernatant, serum, and plasma. It is analytically validated with ready-to-use reagents.

Tel: 858-768-5800

Materials Provided:

Description	Quantity (1 plate)	Quantity (5 plates)	Volume (per bottle)	Part #
Anti-human IL-31 Precoated 96-well Strip Microplate	1 plate	5 plates		77651
Human IL-31 Detection Antibody	1 bottle	5 bottles	12 mL	77652
Human IL-31 Standard	1 vial	5 vials	lyophilized	78886
Avidin-HRP	1 bottle	5 bottles	12 mL	77897
Assay Buffer A	1 bottle	5 bottles	25 mL	78232
Wash Buffer (20X)	1 bottle	5 bottles	50 mL	78233
Substrate Solution F	1 bottle	5 bottles	12 mL	79132
Stop Solution	1 bottle	5 bottles	12 mL	79133
Plate Sealers	4 sheets	20 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 Strips	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 can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP	
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 and is corrosive. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

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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°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 20 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 20 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:

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 Human IL-31 Standard by adding the volume of Assay Buffer A to make a 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.
3. In general, samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer A as the sample diluent.

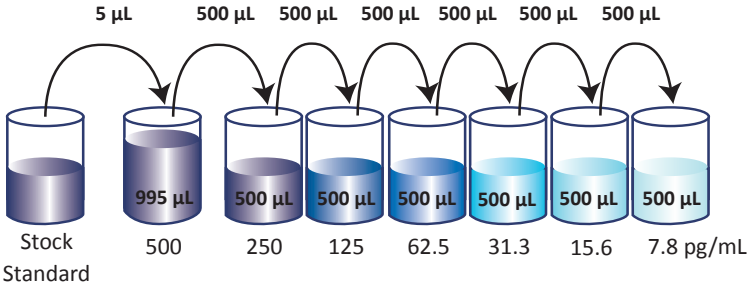
Assay Procedure:

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 1,000 µL of the 500 pg/mL top standard by diluting 5 µL of the standard stock in 995 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 500 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the Human IL-31 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).



4. Wash the plate 4 times with 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on clean, absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Buffer A to each well that will contain either standard dilutions or samples.

6. Add 50 µL of standard dilutions or samples to the appropriate wells.

7. Seal the plate with a Plate Sealer included in the kit and incubate the plate **overnight** for 18-20 hours at 2-8˚C. Do not shake the plate for this step.

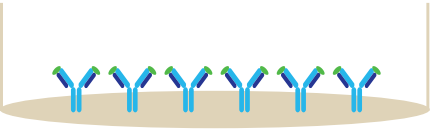
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of Human IL-31 Detection Antibody solution to each well, seal and the plate incubate at room temperature for 2 hour while shaking.

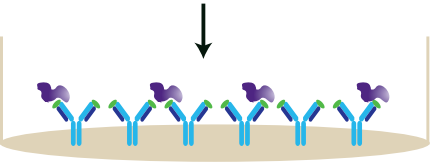
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP solution to each well, seal and incubate the plate at room temperature for 30 minutes while shaking.
12. Discard the contents of the plate into a sink, then wash 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.
13. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 15 minutes in the dark. Wells containing human IL-31 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm immediately. 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

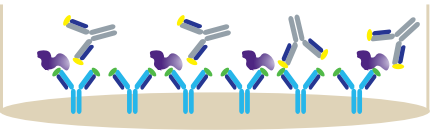
1. Wash 4 times.
   Add 50 µL Assay Buffer A to standard wells and sample wells



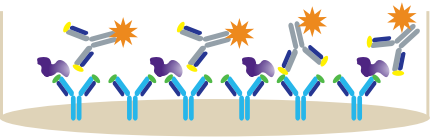
2. Add 50 µL diluted standards or samples,
   Incubate 18-20 hrs, 2-8^{0}C



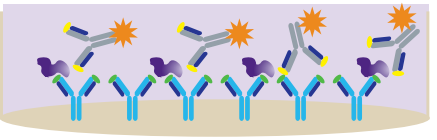
3. Wash 4 times
   Add 100 µL Detection Antibody solution
   Incubate 2 hr, RT, shaking



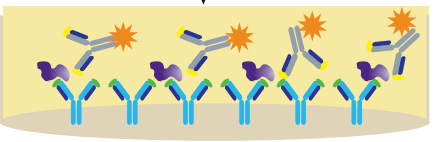
4. Wash 4 times
   Add 100 µL Avidin-HRP solution
   Incubate 30 mins, RT, shaking



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



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

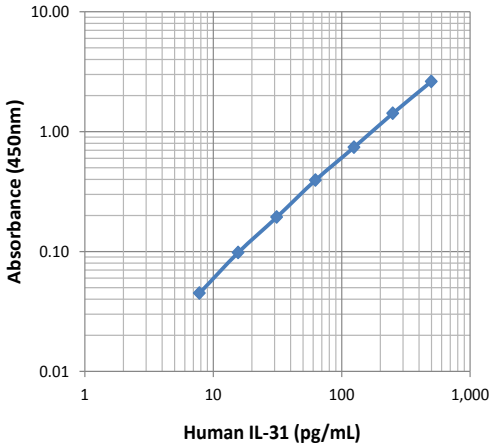
Calculation of Results:

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 protein concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown protein 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 protein 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 recombinant proteins, each at 50 ng/mL.

Human-Proteins	IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-13, IL-27, TNF-α, EPO
Mouse Proteins	IL-2, IL-3, IL-4, IL-6, IL-10, IL-11, IL-27, IL-17A, IL-17F

Sensitivity: The minimum detectable concentration is 1.1 pg/mL ± 0.4 pg/mL (n=8).

Recovery: Recombinant human IL-31 at concentrations of 250, 125, and 62.5 pg/mL, was spiked into the following human samples, then analyzed with the LEGEND MAX™ Human IL-31 ELISA Kit.

Sample Type	N	% Recovery
Serum	4	87
EDTA Plasma	2	78
Heparin Plasma	4	94
Citrate Plasma	4	93

Linearity: Recombinant human IL-31 was spiked into human serum and plasma samples to produce samples within the dynamic range, and then assayed with the kit to determine the dilutional linearity up to 8-fold dilutions. Alternatively, stimulated human PBMC contaning high levels of IL-31 was diluted 2-fold with Assay Buffer A and assayed up to 16-fold dilutions.

Sample Type	N	% Linearity
Serum	4	100
EDTA Plasma	4	114
Heparin Plasma	4	111
Citrate Plasma	4	105
Stimulated PBMC	1	93

Intra-Assay Precision: Two serum samples was spiked with different recombinant human IL-31 concentrations were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	279.5	76.4
Standard Deviation	15.9	6.0
% CV	5.7	7.8

Inter-Assay Precision: Two human samples was spiked with different concentrations of recombinant human IL-31 were assayed in four independent assays by different operators.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	262	72.8
Standard Deviation	20.1	3.8

Biological Samples:
Normal Ranges: Serum, plasma (Heparain, EDTA, and Citrate) samples from human donors were tested for endogenous human IL-31.

Sample Type	N	% Detectable	Min (pg/mL)	Max (pg/mL)	Median (pg/mL)
Serum	20	10	ND	20	13.3
EDTA	20	15	ND	36	23.3
Heparin	19	10	ND	18.7	12.1
Citrate	20	10	ND	24.9	21.4

Cell Culture Supernatant - Human PBMC (1 x 10^6 cells/mL) was stimulated with 10 µg/mL plate-coated anti-CD3 antibody plus 1 µg/mL soluble anti-CD28 antibody at 37°C for 48 hours. Cell culture supernatants were collected and analyzed for natural levels of human IL-31. The concentration of human IL-31 was 316 pg/mL in CD3/CD28-stimulated sample and non-detectable in unstimulated samples.

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												

Notes

Notes

Tel: 858-768-5800

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LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
9727 Pacific Heights Blvd.
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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