BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Mouse LIF

Cat. No. 445107 1 Plate

445108 5 Plates

ELISA Kit for Accurate Quantitation of Mouse LIF from Cell Culture Supernatant, Serum, and Plasma

BioLegend, Inc.
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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.

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Introduction:

LIF stands for Leukemia Inhibitory Factor. It belongs to the IL-6 family of cytokines, and is a variably glycosylated, secreted protein with a mass of 38-67kD. The mature mouse form has 180 amino acids, and contains six conserved cysteines which form three intramolecular disulfide bonds. The protein itself forms a four-helix bundle fold. There are two isoforms of mouse LIF formed by alternative splicing that differ by their localization signals. Mouse LIF shares 92% and 78% amino acid identity with rat LIF and human LIF, respectively.

LIF was originally named for its observed function to promote differentiation of mouse M1 leukemic myeloid cell line into macrophages. It often has contradictory effects within different contexts, either stimulating cell proliferation, or inducing differentiation and apoptosis. LIF has been an important laboratory tool to help maintain stem cell pluripotency. It is involved in the mechanisms of embryonic implantation, inflammatory disorders such as arthritis, immunomodulation, carcinogenesis, and neoplasia-associated cachexia.

LIF binds to both LIF Rα and gp130 on the cell surface to form a heterotrimeric complex, which initiates cell signalling through the JAK-STAT pathway. LIF can also activate the MAPK, PI3K, and p53 signalling pathways. Soluble forms of both binding partners do exist in circulation, and potentially serve to further regulate LIF action.

BioLegend's LEGEND MAX™ Mouse LIF ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a goat polyclonal anti-mouse LIF capture antibody. The Detection Antibody is a biotinylated goat polyclonal anti-mouse LIF antibody. This kit is specifically designed for the accurate quantitation of mouse LIF 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-mouse LIF Pre-coated 96-well Strip Microplate	1 plate	5 plates		76292
Mouse LIF Detection Antibody	1 bottle	5 bottles	12 mL	76293
Mouse LIF Standard	1 vial	5 vials	lyophilized	76295
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.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate Strips</td><td>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.</td></tr>
<tr><td>Standard</td><td>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.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="6">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP</td></tr>
<tr><td>Assay Buffer A</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

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 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 tubes containing an appropriate anticoagulant, and ensure a clean stick with no hemolysis. Centrifuge immediately for 10 minutes at 1,000 x *g*. Carefully remove supernatant and assay immediately or store 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 Mouse LIF Standard by adding the volume of Assay Buffer A to make a 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.
3. For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilutions are necessary, samples should be diluted in Assay Buffer A.
4. It is recommended that serum or plasma samples be analyzed with no dilution.

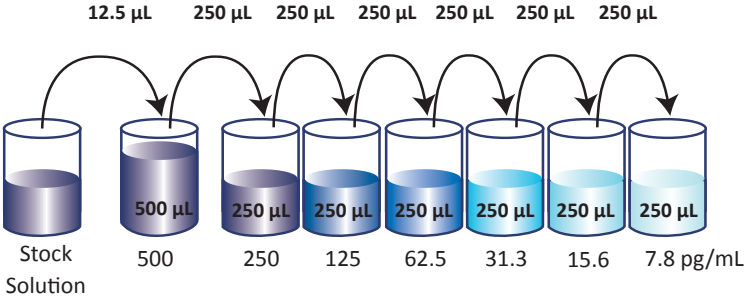
Assay Procedure:

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 500 pg/mL top standard by diluting 12.5 µL of the standard stock solution in 487.5 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 mouse LIF 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 properly diluted samples to the appropriate wells.

7. 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.

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

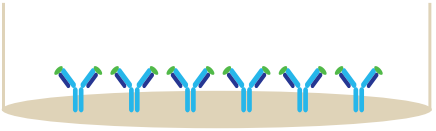
9. Add 100 µL of Mouse LIF Detection Antibody solution to each well, seal the

plate and incubate at room temperature for 1 hour while shaking.

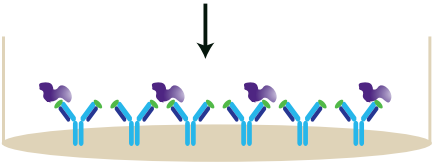
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
12. Discard the contents of the plate into a sink, then wash 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 30 minutes in the dark. Wells containing mouse LIF 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

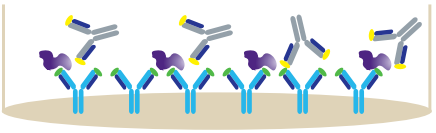
1. Wash 4 times.
   Add 50 µL Assay Buffer A.



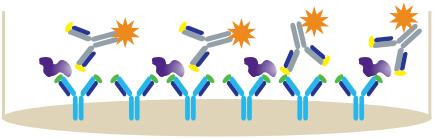
2. Add 50 µL diluted standards or samples.
   Incubate 2 hrs, RT, shaking.



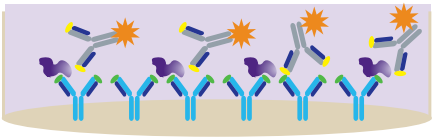
3. Wash 4 times.
   Add 100 µL Detection Antibody solution.
   Incubate 1 hr, RT, shaking.



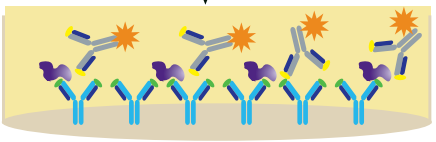
4. Wash 4 times.
   Add 100 µL Avidin-HRP solution.
   Incubate 30 mins, RT, shaking.



5. Wash 5 times.
   Add 100 µL Substrate Solution F.
   Incubate 30 mins, RT, in the dark.



6. Add 100 µL Stop Solution.



7. Read absorbance at 450 nm and 570 nm.

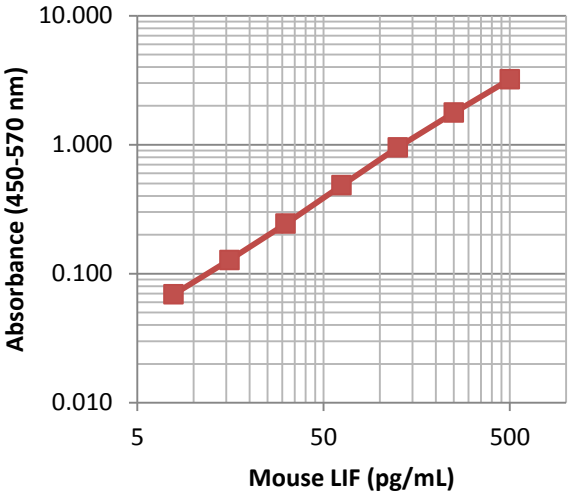
Calculation of Results:

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.

Category	Sample
Mouse Proteins	LIF R, gp130, CCL17 (TARC), CCL2 (MCP-1), CXCL10 (IP-10), CXCL11 (ITAC), CXCL12 (SDF-1β), CXCL9 (MIG), IFN-β1, IFN-γ, IL-12 (p70), IL-17A, IL-17A/F Heterodimer, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-33, IL-34, TNF-α, GM-CSF, MMP-9 (Gelatinase B), NGAL (Lipocalin-2)

This kit cross-reacts with recombinant human LIF (50 ng/mL) at 0.7% relative to recombinant mouse LIF.

Sensitivity: The minimum detectable concentration is 1.6 pg/mL ± 0.8 pg/mL (Mean ± 2 SDs) (n=11).

Recovery: Recombinant mouse LIF, at concentrations of 250, 62.5, and 15.6 pg/mL, was spiked into the following mouse samples, then analyzed with the LEGEND MAX™ Mouse LIF ELISA Kit.

Sample Type	N	% Recovery
Serum	4	86
EDTA Plasma	4	94
Heparin Plasma	4	87
Citrate Plasma	4	91

Linearity: Recombinant mouse LIF was spiked at 250 pg/mL into mouse 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 mouse splenocytes with high levels of LIF were diluted 10-fold with Assay Buffer A and assayed up to 1280-fold dilutions.

Sample Type	N	% Linearity
Serum	4	113
EDTA Plasma	4	86
Heparin Plasma	4	112
Citrate Plasma	4	97
Stimulated Splenocytes	1	98

Intra-Assay Precision: Two samples of mouse serum spiked with two different amounts of stimulated splenocytes were tested with 8 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	8	8
Mean Concentration (pg/mL)	68	12.7
Standard Deviation	1.7	0.3
% CV	2.5	2.5

Inter-Assay Precision: Two samples of mouse serum spiked with two different amounts of stimulated splenocytes were tested in four or three independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	3
Mean Concentration (pg/mL)	68.1	13.3
Standard Deviation	4.8	0.7
% CV	7.0	5.4

Biological Samples:

Normal Ranges - Serum and plasma (Heparain, EDTA, and Citrate) samples of four mouse strains (C57BL/6, BALB/c, Swiss Webster, and CD-1) were pooled from a minimum of ten mice each. All samples were tested for endogenous LIF.

Sample Type	N	% Detectable	Min. (pg/mL)	Max. (pg/mL)	Median (pg/mL)
Serum	4	0	ND*	ND*	ND*
EDTA Plasma	4	25	ND*	17	0
Heparin Plasma	4	25	ND*	2	0
Citrate Plasma	4	25	ND*	3	0

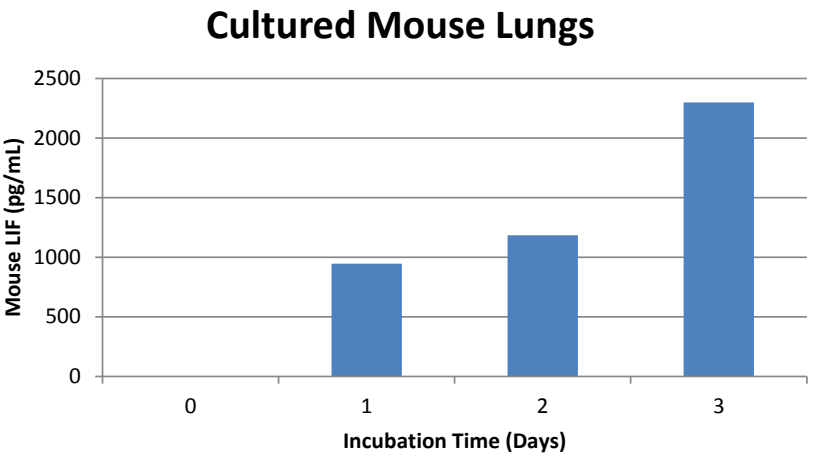
**Not Detectable*

Cell Culture Supernatant - Mouse splenocytes were isolated and plated in a 12-well plate at ~2 x 10^6 cells/mL in 10% FBS/RPMI. Cells were stimulated with CD3 and CD28 (both at 2 µg/mL), and supernatant was removed at day 1 and day 4 after stimulation and assayed for mouse LIF, both neat and after a 20-fold dilution in Assay Buffer A.

Stimulation	Time	pg/mL
CD3/CD28	Day 1	370
CD3/CD28	Day 4	4124
Unstimulated	Day 4	ND*

**Not Detectable*

Tissue Culture Supernatant - The lungs from one mouse were cut into smaller pieces and cultured in 10% FBS/RPMI. Supernatants were removed at 0, 1, 2, and 3 days and assayed for mouse LIF both, neat and after a 20-fold dilution in Assay Buffer A.



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												

Notes

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Notes

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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