BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Mouse IL-10

Cat. No. 431417

ELISA Kit for Accurate Quantitation of Mouse IL-10 from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

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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:

Mouse IL-10 (Interleukin 10, B cell derived T cell growth factor, B-TCGF, Cytokine synthesis inhibitory factor (CSIF), T-cell growth inhibitory factor (TGIF) was originally described as Cytokine Synthesis Inhibitory Factor (CSIF) due to its ability to inhibit cytokine production by T_H1 clones. IL-10 is expressed in activated $CD8^+$ and $CD4^+$ T cells, activated monocytes, mast cells, and Ly-1 B cells. IL-10 shares over 80% sequence homology with the Epstein-Barr virus protein BCRFI. The functions of IL-10 include inhibition of macrophage-mediated cytokine synthesis, suppression of the delayed type hypersensitivity response, and stimulation of the T_H2 cell response, which results in elevated antibody production.

The BioLegend LEGEND MAX™ Mouse IL-10 ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal rat anti-mouse IL-10 capture antibody. The detection antibody is a biotinylated monoclonal rat anti-mouse IL-10 antibody. This kit is specifically designed for the accurate quantitation of mouse IL-10 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-mouse IL-10 Pre-coated 96-well Strip Microplate	1 plate		79876
Mouse IL-10 Detection Antibody	1 bottle	12 mL	79877
Mouse IL-10 Standard	1 vial	lyophilized	79067
Matrix C (for serum and plasma samples only)	1 vial	lyophilized	78316
Avidin-HRP D	1 bottle	12 mL	78237
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	1 pack		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
- 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 wells</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 rowspan="2">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.</td></tr>
<tr><td>Matrix C</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 D</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/support/#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 2N sulfuric 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°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 citrate, heparin or 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.

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 2 mL of deionized water into the vial and allow the reconstituted Matrix C to sit at room temperature for 15 minutes, then vortex to mix completely.
3. Reconstitute the lyophilized Mouse IL-10 Standard by adding the volume of Assay Buffer A to make the 20 ng/mL standard stock solution (Refer toLEGEND 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.
4. For measuring cell culture supernatant samples, if dilutions are required, use Assay Buffer A for diluting.

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5. For measuring serum or plasma samples, a 2-fold dilution of the samples with Assay Buffer A is required. If further dilution is necessary, samples should be further diluted with Matrix C.

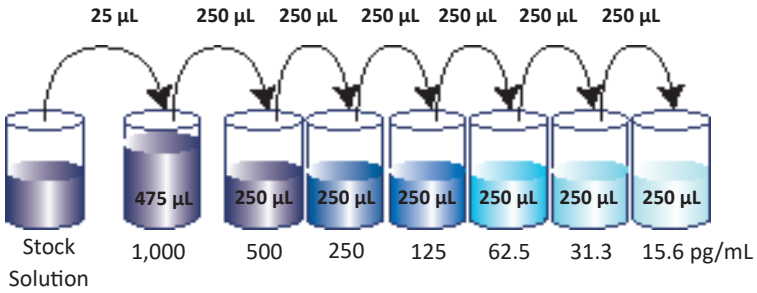
Assay Procedure:

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 1,000 pg/mL top standard by diluting 25 µL of the standard stock solution in 475 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 1,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the mouse IL-10 standard concentrations in the tubes are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL and 15.6 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 samples.

b) Add 50 µL of standard dilutions to the wells containing Matrix C. Add 50 µL 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 µL of Mouse IL-10 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 µL 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 µL of Substrate Solution F to each well and incubate for 30 minutes in the dark. Wells containing mouse IL-10 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

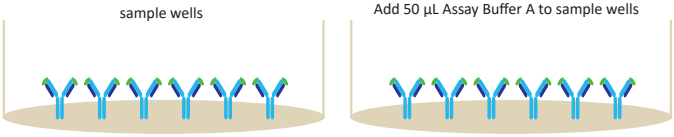
13. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

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

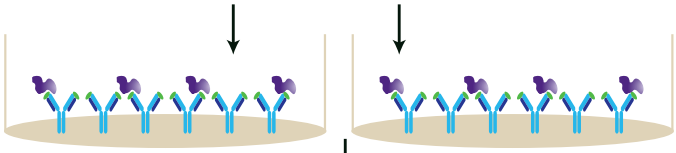
1. **For measuring cell culture supernatant:**
   Wash 4 times
   Add 50 µL Assay Buffer A to standard and sample wells

   For measuring serum or plasma:
   Wash 4 times
   Add 50 µL Matrix C to standard wells
   Add 50 µL Assay Buffer A to sample wells

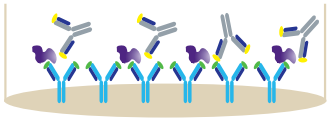


2. Add 50 µL diluted standards to standard wells
   Add 50 µL samples to sample wells
   Incubate 2 hr, RT, shaking

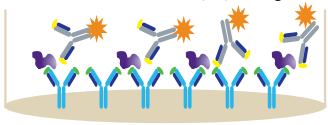
   Add 50 µL diluted standards to standard wells
   Add 50 µL of pre diluted samples to sample wells
   Incubate 2 hr, RT, shaking



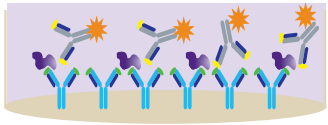
3. Wash 4 times
   Add 100 µL Detection Antibody solution
   Incubate 1 hr, RT, shaking



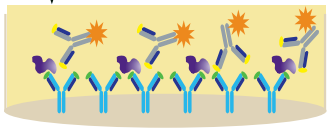
4. Wash 4 times
   Add 100 µL Avidin-HRP D solution
   Incubate 30 min, RT, shaking



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



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

Calculation of Results:

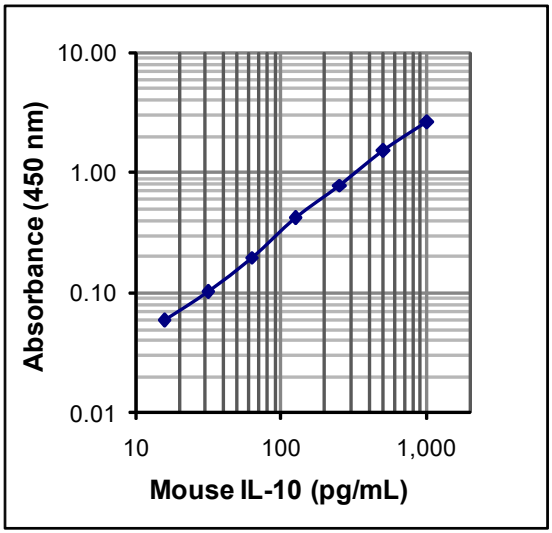
The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate 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.

Because mouse serum/plasma samples have been diluted prior to the assay, their measured concentrations must be multiplied by the dilution factor.

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 human IL-10 and the following recombinant cytokines/chemokines, each at up to 50 ng/mL.

Mouse	IFGF-Basic, IL-3, IL-12, IL-12 p40, IL-13, IL-15, IL-17A/F, IL-17F, IL-1β, IL-2, IL-25, IL-27, IL-33, IL-34, IL-4, IL-5, IL-6, IL-7, Isthmin, SCF, TNF-α, TRANCE, VEGF
Human	IL-2, IL-4, IL-6, MCP-1
Rat	IL-4, MCP-1, TNF-α

Sensitivity: The minimum detectable concentration of IL-10 is 2.7 pg/mL.

Recovery: IL-10 (500, 125 and 31 pg/mL) was spiked into 6 mouse serum samples, and then analyzed with a LEGEND MAX™ Mouse IL-10 ELISA kit. On average, 90.8% of the cytokine was recovered from the serum samples.

Linearity: Five 2 fold diluted murine serum samples, each from a different strain, were spiked with high concentrations of IL-10 then diluted with Matrix C to produce samples with concentrations within the dynamic range and then assayed. On average, 101.6 % of the expected cytokine concentration was detected from the serum samples.

Intra-Assay Precision: Sixteen replicates of each of two samples containing different IL-10 concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	452.9	119.9
Standard Deviation	18.4	4.8
% CV	4.1	2.7

Inter-Assay Precision: Two samples containing different concentrations of IL-10 were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	474.9	114.7
Standard Deviation	16	5.6
% CV	3.4	4.9

Biological Samples:

Serum/Plasma - Normal mouse serum samples (n = 6), each from a different strain, were assayed for basal levels of mouse IL-10. All samples assayed as undetectable for mouse IL-10.

Cell Culture Supernatant - Mouse splenocytes (1 x 10^6 cells/mL) were cultured with 10 µg/mL plate-coated anti-CD3 antibody and 1 µg/mL soluble anti-CD28 antibody at 37°C for 2 days. Cell culture supernatants were collected and assayed for levels of natural mouse IL-10. The resulting IL-10 concentration averaged 10,640 pg/mL in anti-CD3/anti-CD28-stimulated samples and there was no detectable IL-10 in unstimulated 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												

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