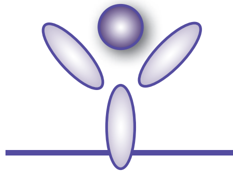
LEGEND MAX™

ELISA Kit with Pre-coated Plates



Mouse CXCL13 (BLC)

Cat. No.	441907	1 Plate
	441908	5 Plates

ELISA Kit for Accurate Quantitation of Mouse CXCL13 (BLC) 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.

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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Introduction:

CXCL13, also known as B-Lymphocyte Chemoattractant (BLC), or B Cell-Attracting chemokine 1 (BCA-1), is a small cytokine belonging to the CXC chemokine family. As a strong chemoattractant for B cells, it is highly expressed in the follicles of Peyer's patches, spleen, and lymph nodes. It is also expressed by T follicular helper cells, dendritic cells, and stromal cells (including marginal reticular cells (MRCs) and follicular dendritic cells (FDCs)) in B cell areas of secondary lymphoid tissue. CXCL13 and its receptor CXCR5 regulate compartmentalization of B- and T-cells in secondary lymphoid organs. During the migration in the follicle, B cells are in contact with FDCs, MRCs, and cells localized around the follicle perimeter, such as sinus-associated macrophages and T cell zone associated DCs. Subsequent to the migration through the FDC network, B cells travel through the T cell zone-proximal follicle. In this migration, CCL21, CCL19, CCR7 and CXCR5 are involved. CXCL13, CCL19, and CCL21 take part in the formation of tertiary lymphoid tissues in chronic antigen-induced arthritis. In CXCL13 deficient mice, B-cells fail to organize in polarized follicular clusters and instead appeare as a ring of cells at the perimeter of T-cell areas.

CXCL13 is highly expressed at sites of new lymphoid tissue formation in a variety of chronic inflammatory conditions. High expression of CXCL13 has been linked to lupus, Helicobacter pylori-associated gastritis, multiple sclerosis, rheumatoid arthritis, and the allergic airway inflammatory process.

LEGEND MAX™ Mouse CXCL13 (BLC) ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a rat monoclonal anti-mouse CXCL13 antibody. The detection antibody is a biotinylated rat monoclonal anti-mouse CXCL13 antibody. This kit is specifically designed for the accurate quantitation of mouse CXCL13 from cell culture supernatant, serum and plasma. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity (1 plate)	Quantity (5 plates)	Volume (per bottle)	Part #
Anti-Mouse CXCL13 Pre-coated 96 well Strip Microplate	1 plate	5 plates		77253
Mouse CXCL13 Detection Antibody	1 bottle	5 bottles	12 mL	77254
Mouse CXCL13 Standard	1 vial	5 vials	lyophilized	77256
Avidin-HRP	1 bottle	5 bottles	12 mL	77897
Assay Buffer B	1 bottle	5 bottles	25 mL	79128
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 B</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.

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. *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 mix until dissolved.
2. Reconstitute the lyophilized Mouse CXCL13 Standard by adding the volume of Assay Buffer B 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.
3. For cell culture supernatants samples, the end user may need to determine the dilution factors in a preliminary experiment. Samples should be diluted in Assay Buffer B if dilutions are necessary. Fresh cell culture media should be used as the control.
4. It is recommended that serum and plasma samples be diluted 1:10 with Assay Buffer B prior to analysis. If further dilution is needed, samples should be diluted with Assay Buffer B. For example, to make a 10 fold dilution of serum/plasma samples in a final volume of 150 µL, dilute 15 µL of serum/plasma samples in 135 µL Assay Buffer B.

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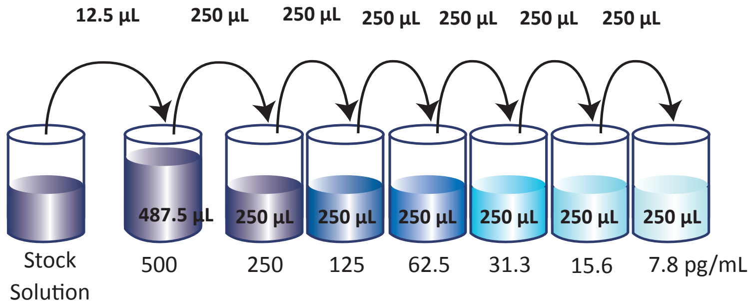
Assay Procedure:

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. 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 B. Perform six two-fold serial dilutions of the 500 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the mouse CXCL13 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 B 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 clean, absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Buffer B 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 CXCL13 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 for 10 minutes in the dark. Wells containing mouse CXCL13 should turn blue in color with intensity proportional to 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 well 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.

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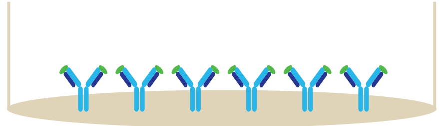
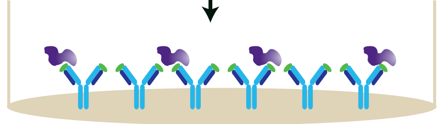
Assay Procedure Summary

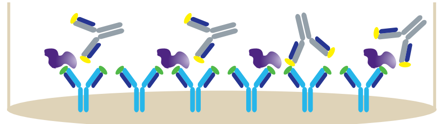
1. Wash 4 times
Add 50 µL Assay Buffer B



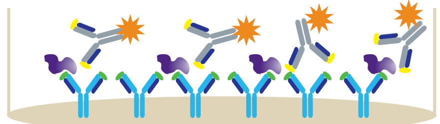
2. Add 50 µL diluted standards or samples
Incubate 2 hr, RT, shaking



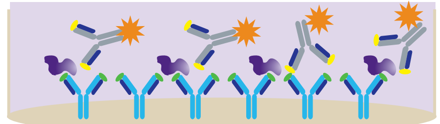
3. Wash 4 times,
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



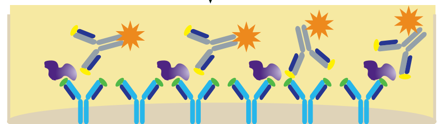
4. Wash 4 times
Add 100 µL Avidin-HRP solution
Incubate 30 min, RT, shaking



5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 10 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

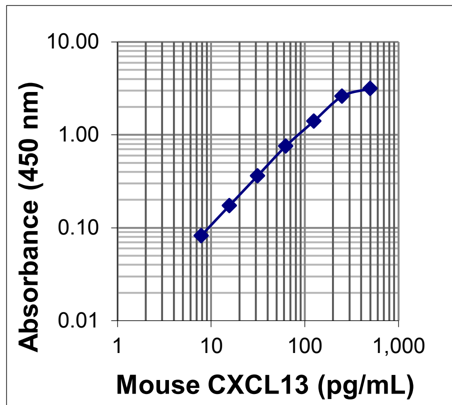
Calculation of Results:

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 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 cytokines/chemokines, each at 50 ng/mL.

Mouse	CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL7, CXCL9, CXCL10, CXCL11, CXCL12, CXCL14, CXC17, CX3CL1, CCL1, CCL2, CCL8, CCL9, CCL11, CCL12, CCL17, CCL20.
Human	CCL1, CCL2, CCL3, CCL5, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL16, CXCL17 CX3CL1.

Sensitivity: The average minimum detectable concentration of mouse CXCL13 is 1.0 ± 0.4 pg/mL. (n=4).

Recovery: Three levels of recombinant mouse CXCL13 (250 pg/mL, 62.5 pg/mL and 15.6 pg/mL) were spiked into four strains of pooled mouse serum samples, and analyzed with the LEGEND MAX™ Mouse CXCL13 (BLC) ELISA Kit. On average, 108% of the cytokine was recovered from serum samples.

Linearity: Four strains of pooled mouse serum samples containing high concentration of mouse CXCL13 were diluted with Assay Buffer B to produce sample concentrations within the dynamic range of the assay. On average, 99% of the expected cytokine was detected from serum samples.

Intra-Assay Precision: Two samples with different concentration of recombinant mouse CXCL13 were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	131.6	37.2
Standard Deviation	2.7	0.8
% CV	1.7	2.2

Inter-Assay Precision: Two samples with different concentrations of recombinant mouse CXCL13 were assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	122.8	34.8
Standard Deviation	9.3	2.8
% CV	7.6	7.8

Biological Samples:

Serum or Plasma

Serum or plasma samples of four strains (BALB/c, C57BL/6, CD-1 and Swiss Webster) were pooled from a minimum of ten mice each and tested for endogenous CXCL13. The concentrations (pg/mL) measured are shown below:

	Serum	EDTA	Citrate	Heparin
Detectable %	100	100	100	100
Mean (pg/mL)	1324	871	521	671
Max (pg/mL)	3022	1429	854	1072
Min (pg/mL)	365	236	247	104

Cell Culture Supernatant

Mouse splenocytes at a concentration of 2 x 10^6 cells/mL were stimulated with 10 μg/mL of plate-coated anti-CD3 antibody in addition to 1 μg/mL of soluble anti-CD28 antibody at 37°C for 1 day. The cell culture supernatant was collected and assayed for the concentration of natural mouse CXCL13. The concentration of mouse CXCL13 was 31 pg/mL in anti-CD3 and anti-CD28 double-stimulated samples, and undetectable in unstimulated sample.

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Troubleshooting Guide:

<table>
<tr><th>Problem</th><th>Probable Cause</th><th>Solution</th></tr>
<tr><td rowspan="3">High Background</td><td>Background wells were contaminated</td><td>Avoid cross-well contamination by using the provided plate sealers.
Use multichannel pipettes and change tips between pipetting samples and reagents.</td></tr>
<tr><td>Insufficient washes</td><td>Increase number of washes.
Increase soaking time between washes prior to addition of substrate solution.</td></tr>
<tr><td>TMB Substrate Solution was contaminated</td><td>TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.</td></tr>
<tr><td rowspan="5">No or poor signal</td><td>Detection Antibody, Avidin-HRP or Substrate solution were NOT added</td><td rowspan="2">Rerun the assay and follow the protocol.</td></tr>
<tr><td>Wrong reagent or reagents were added in wrong sequential order</td></tr>
<tr><td>Insufficient plate agitation</td><td>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.</td></tr>
<tr><td>The wash buffer contains Sodium Azide (NaN3)</td><td>Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.</td></tr>
<tr><td>Incubations were done at an inappropriate temperature, timing or without agitation</td><td>Rerun the assay and follow the protocol.</td></tr>
<tr><td rowspan="3">Low or poor standard curve signal</td><td>The standard was incorrectly reconstituted or diluted</td><td>Adjust the calculations and follow the protocol.</td></tr>
<tr><td>Standard was inappropriately stored</td><td>Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Reagents added to wells with incorrect concentrations</td><td>Check for pipetting errors and the correct reagent volume.</td></tr>
</table>

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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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.**
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

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