BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human CXCL10/IP-10

Cat. No. 439907

ELISA Kit for Accurate Quantitation of Human CXCL10/IP-10 from Cell Culture Supernatant, Urine, Saliva, 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:

IP-10 (interferon-gamma inducible protein 10 kD), also known as CXCL10, is a glutamic acid-leucine-arginine motif negative chemokine structurally and functionally related to MIG (CXCL9) and ITAC (CXCL11). CXCL10/IP-10 is induced in a variety of cells in response to interferon-γ and lipopolysaccharide. These cell types include monocytes, macrophages, fibroblasts, and epithelial cells. CXCL10/IP-10 chemoattracts $CD4^+$ and $CD8^+$ T cells, NK, and NKT cells via binding to its receptor CXCR3, which is shared with MIG and ITAC.

CXCL10/IP-10 inhibits neovascularization in tumors and in wound healing *in vivo*. It also has anti-proliferative effects on endothelial cells *in vitro*, and angiostatic and antitumor effects *in vivo*. It has been suggested that the anti-proliferative effect of CXCL10/IP-10 in endothelial cells is CXCR3-independent and that it is mediated through glycosaminoglycan interaction. CXCL10/IP-10 also possesses antimicrobial activity against *E. coli* and *L. monocytogenes*, and both the spore and bacillus forms of *B. anthracis*. It has been reported that CXCL10/IP-10 may be important in the pathogenesis of chronic HCV infection, and the circulating CXCL10/IP-10 concentration was increased in the early and subclinical stage of Type I diabetes patients. CXCL10/IP-10 was significantly up-regulated in the salivary glands of patients with Sjögren's Syndrome. It also has been reported that CXCL10/IP-10 was increased in the urine of patients with pulmonary diseases in the absence of renal dysfunction.

BioLegend's LEGEND MAX™ Human CXCL10/IP-10 ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal mouse anti-human CLCX10/IP-10 capture antibody. The detection antibody is a biotinylated polyclonal goat anti-human CXCL10/IP-10 antibody. This kit is specifically designed for the accurate quantitation of human CXCL10/IP-10 from cell culture supernatant, urine, saliva, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-Human CXCL10/IP-10 Pre-coated 96-well Strip Microplate	1 plate		79905
Human CXCL10/IP-10 Detection Antibody	1 bottle	12 mL	79906
Human CXCL10/IP-10 Standard	1 vial	lyophi-lized	79949
Matrix C (for serum and plasma samples only)	1 vial	lyophi-lized	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	4 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 wells	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 or Matrix C can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Matrix C	
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP D	
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 urine, saliva, 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°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.

Urine: 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.

Saliva: Centrifuge all samples at 10,000 x *g* at 4°C for 20 minutes and collect supernatants. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use. Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

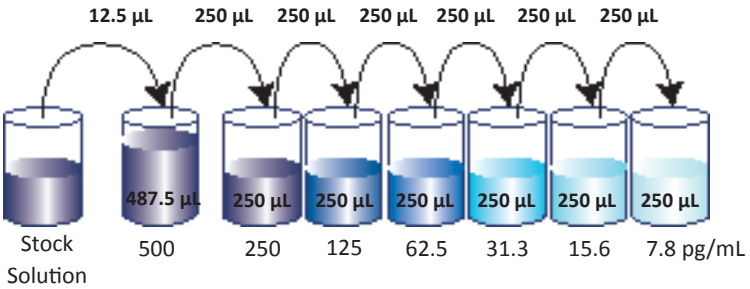
1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.
2. Reconstitute the lyophilized Human CXCL10/IP-10 Standard by adding the volume of Assay Buffer A to make the 20 ng/mL standard stock 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. 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.
4. In general, serum or plasma samples are analyzed without dilutions. However, if dilutions are required, use Matrix C as the sample diluent.
5. Saliva samples require at least 5-fold dilutions. Use Assay Buffer A as the sample diluent.
6. For cell culture supernatant, it is recommend to run a number of dilutions to determine the optimal dilution factor. Use the control culture medium or Assay Buffer A as the sample diluent.
7. In general, urine samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer A as the sample diluent.

Assay Procedure:

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 human CXCL10/IP-10 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 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 cell culture supernatant, urine, or saliva samples:**

   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 standard dilutions and 50 µL of Assay Buffer A to each well that will contain samples.

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

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.

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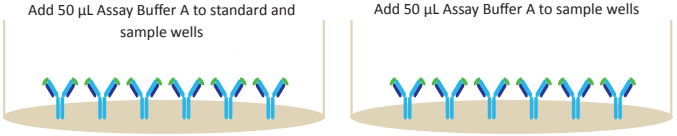
7. Discard the plate contents into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
8. Add 100 µL of Human CXCL10/IP-10 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
9. Discard the contents of the plate into a 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. Incubation 20 minutes for serum/plasma samples, or 15 minutes for cell culture supernatant, urine, and saliva samples in the dark. Wells containing human CXCL10/IP-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

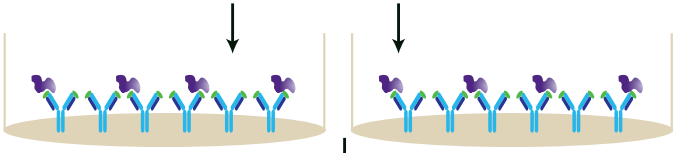
1. **For measuring cell culture supernatant, urine, or saliva:**
   Wash 4 times
   Add 50 µL Assay Buffer A to standard and sample wells

   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

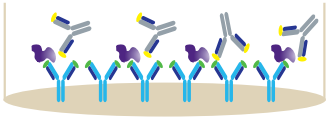


2. Add 50 µL diluted standards to standard wells
   Add 50 µL samples to sample wells
   Incubate 2 hr, RT, shaking

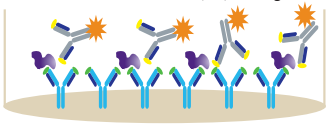
   Add 50 µL diluted standards to standard wells
   Add 50 µL samples to sample wells
   Incubate 2 hr, RT, shaking



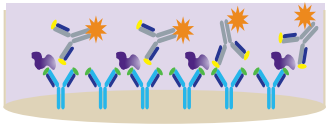
3. Wash 4 times
   Add 100 µL Detection Antibody solution
   Incubate 1 hr, RT, shaking



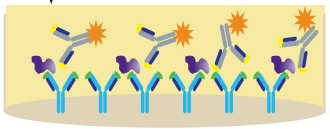
4. Wash 4 times
   Add 100 µL Avidin-HRP D solution
   Incubate 30 min, RT, shaking



5. Wash 5 times
   Add 100 µL Substrate Solution F
   Incubate 15-20 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

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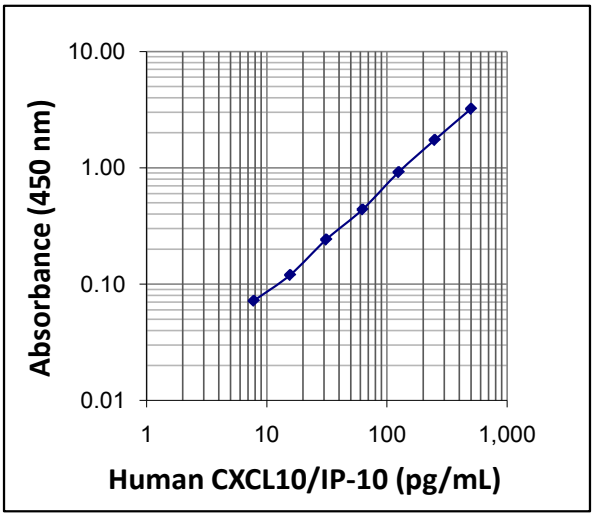
Calculation of Results:

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 analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte 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 analyte 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: There was no detectable cross-reactivity when the following recombinant cytokines/chemokines were tested at up to 50 ng/mL.

Human	CCL2, CCL17, CCL19, CCL21, CXCL1, CXCL2, CXCL5, CXCL9, CXCL11, CXCL12, CXCL13, G-CSF, IL-1α, IL-1β, IL-3, IL-5, IL-7, IL-10, IL-12, IL-13, IL-17A, IL-17F, IL-21, IL-22, IL-33, IL-34, TGF-β1, TNF-β
Mouse	CCL2, CCL8, CCL17, CCL20, CXCL1, CXCL2, CXCL5, CXCL9, CXCL10/IP-10, CXCL11, CXCL12, GM-CSF, IL-1α, IL-3, IL-4, IL-5, IL-6, IL-12, IL-17A/F, IL-17E, IL-17F, IL-22, IL-23, IL-33, TNF-α, VEGF

Sensitivity: The average minimum detectable concentration of CXCL10/IP-10 is 1.38 pg/mL.

Recovery: Two concentrations of CXCL10/IP-10 were spiked into human urine, saliva, serum/plasma samples, and then analyzed with the LEGEND MAX™ Human CXCL10/IP-10 ELISA Kit. On average, 91.9%, 85.7%, and 95.7% of the CXCL10/IP-10 was recovered from the urine, saliva, and serum/plasma samples, respectively.

Linearity: Human urine, saliva, and serum/plasma samples with high concentrations of CXCL10/IP-10 were diluted with Assay Buffer A or Matrix C to produce samples with values within the dynamic range and then assayed with the LEGEND MAX™ Human CXCL10/IP-10 ELISA Kit to determine the linearity of dilution. On average, 101%, 91.5%, and 94.4% of the expected IP-10 was detected from the diluted urine, saliva, and serum/plasma samples, respectively.

Intra-Assay Precision: Two samples with varying concentration of CXCL10/IP-10 were assayed with sixteen replicates of each.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	49.0	161.0
Standard Deviation	2.1	7.2
% CV	4.3	4.5

Inter-Assay Precision: Two human CXCL10/IP-10 samples, each with duplicates were assayed in three independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	3	3
Mean Concentration (pg/mL)	125.4	288.7
Standard Deviation	9.7	26.9
% CV	7.7	9.3

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Biological Samples:

Serum/Plasma - Normal human serum/plasma samples (n = 24) were assayed for basal levels of human CXCL10/IP-10. The mean CXCL10/IP-10 value was 145.7 pg/mL, with a range from 40 pg/mL to 344 pg/mL.

Urine - Normal human urine samples (n = 4) were assayed for basal levels of human CXCL10/IP-10. The mean CXCL10/IP-10 value was 61 pg/mL, with a range from 29 pg/mL to 128 pg/mL.

Saliva- Normal human saliva samples (n = 3) were assayed for basal levels of human CXCL10/IP-10. The mean CXCL10/IP-10 value was 1,186 pg/mL, with a range from 925 pg/mL to 1,575 pg/mL.

Cell Culture Supernates - IFN-γ (100 ng/ml) primed human PBMCs at a concentration of $1x10^6$ cells/mL were stimulated with 100 ng/ml LPS at 37°C for 3 days. The cell culture supernatants were collected and assayed for the concentration of natural human CXCL10/IP-10 . The concentration of human CXCL10/IP-10 was 7,176.8 pg/mL in IFN-γ /LPS stimulated samples and 70.2 pg/mL 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.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated absorbance	Standard reconstituted with less volume than required	Reconstitute lyophilized standard with correct volume of solution recommended in the protocol.
	Plate incubation was too long	Decrease incubation time.
	Detection antibody incubation time is too long	Decrease detection antibody incubation time.
	Avidin-HRP incubation time is too long.	Decrease Avidin-HRP incubation time.
	Substrate solution incubation time is too long	Decrease substrate solution incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If samples are below detectable levels, it may be possible to use higher sample volume. Check with technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point.	Samples may require dilution and reanalysis.
High variation in samples and/or standards	Multichannel pipette errors	Calibrate the pipettes.
	Plate washing was not adequate or uniform	Make sure pipette tips are tightly secured. Confirm all reagents are removed completely in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before pipetting.
	Samples may have high particulate matter	Remove the particulate matter by centrifugation.
	Insufficient plate agitation	The plate should be agitated during all incubation steps using an ELISA plate shaker at a speed where solutions in wells are within constant motion without splashing.
	Cross-well contamination	When reusing plate sealers check that no reagent has touched the sealer. Care should be taken when using the same pipette tips used 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

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Notes

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