BioLegend®

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LEGEND MAX™

ELISA Kit

Human FGL2

Cat. No. 436907

ELISA Kit for Accurate Quantitation of Human FGL2 from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

BioLegend, Inc.
biolegend.com

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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.

Introduction:

Fibrinogen-like protein 2 (FGL2), also known as fibroleukin, was first cloned from cytotoxic T lymphocytes and was classified as a member of the fibrinogen superfamily due to its homology (36%) with fibrinogen β and γ chains. In humans, the fgl2 gene encodes a protein of 439 amino acids. Under non-reducing conditions, the molecular mass of the protein is 250-300 kD and in reducing conditions, it has a mass of 64-70 kD. This indicates that FGL2, in its natural state, forms a tetrameric complex. It is postulated that FGL2 has pleiotropic effects and is an important immune regulator of both innate and adaptive responses. In macrophages and endothelial cells, FGL2 is expressed as a membrane-associated protein which acts as a prothrombinase enzyme with the ability to generate thrombin directly from prothrombin. FGL2 also exists as a secreted form, expressed by regulatory T cells, that down-regulates dendritic cell activity and induces B cell apoptosis. The regulatory activity of FGL2 has been implicated in inhibition of allograft rejection, autoimmunity and the pathogenesis of human viral infections.

BioLegend's LEGEND MAX™ Human FGL2 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 FGL2 capture antibody. The detection antibody is a biotinylated monoclonal mouse anti-human FGL2 antibody. This kit is specifically designed for the accurate quantitation of human FGL2 from cell culture supernatant, 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 FGL2 Pre-coated 96-well Strip Microplate	1 plate		78870
Human FGL2 Detection Antibody	1 bottle	12 mL	78876
Human FGL2 Standard	1 vial	lyophilized	78875
Matrix C (for serum and plasma samples only)	1 vial	lyophilized	78316
Avidin-HRP A	1 bottle	12 mL	79131
Assay Buffer B	1 bottle	25 mL	79128
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 A	
Assay Buffer B	
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. *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. 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. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human FGL2 Standard by adding the volume of Assay Buffer B to make the 320 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 8 mL of DI H_2O 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, cell culture supernatant samples are analyzed without dilutions. However, if dilutions are required, use the control culture medium or Assay Buffer B as the sample diluent.
5. For measuring serum or plasma samples, a 5-fold dilution of the samples with Assay Buffer B is required, e.g. add 30 µL of sample to 120 µL of Assay Buffer B.

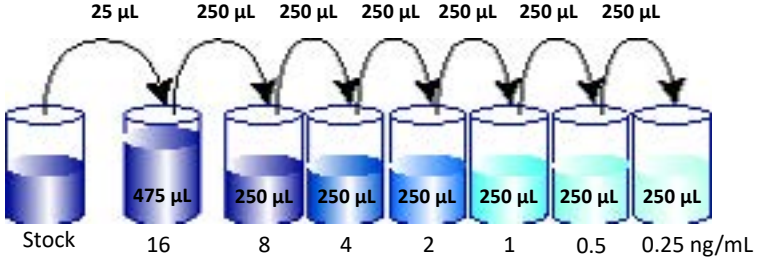
Assay Procedure:

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. For measuring samples of cell culture supernatant:

a) Prepare 500 µL of the 16 ng/mL top standard by diluting 25 µL of the standard stock solution in 475 µL of Assay Buffer B. Perform six two-fold serial dilutions of the 16 ng/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human FGL2 standard concentrations in the tubes are 16 ng/mL, 8 ng/mL, 4 ng/mL, 2 ng/mL, 1 ng/mL, 0.5 ng/mL, and 0.25 ng/mL, respectively. Assay Buffer B serves as the zero standard (0 ng/mL).



b) 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.

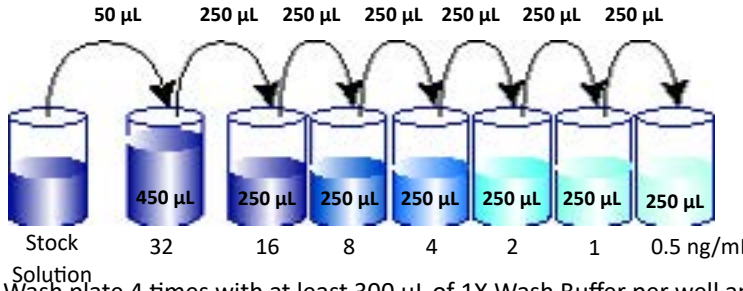
c) Add 50 µL of Assay Buffer B to each well that will contain either standard dilutions or samples.

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

e) 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.

4. For measuring serum or plasma samples:

a) Prepare 500 µL of the 32 ng/mL top standard by diluting 50 µL of the standard stock solution in 450 µL of Assay Buffer B. Perform six two-fold serial dilutions of the 32 ng/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human FGL2 standard concentrations in the tubes are 32 ng/mL, 16 ng/mL, 8 ng/mL, 4 ng/mL, 2 ng/mL, 1 ng/mL, and 0.5 ng/mL, respectively. Assay Buffer B serves as the zero standard (0 ng/mL).



b) 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.

c) Add 50 µL of Matrix C to each well that will contain standard dilutions and 50 µL of Assay Buffer B to each well that will contain samples.

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

e) 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.

5. Discard the plate contents into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 3b or 4b.

6. Add 100 µL of Human FGL2 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 3b or 4b.

8. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

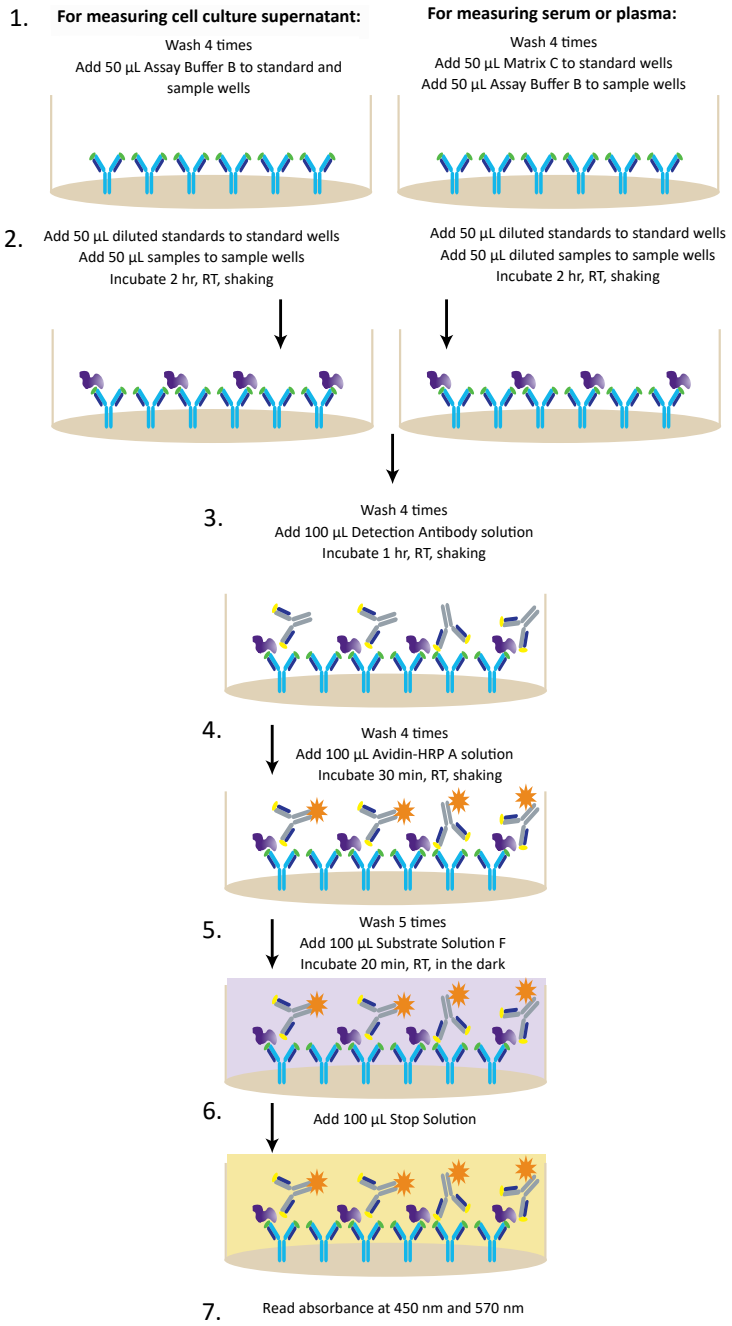
9. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 3b or 4b. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

10. Add 100 µL of Substrate Solution F to each well and incubate for 20 minutes in the dark. Wells containing human FGL2 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

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

12. 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



Calculation of Results:

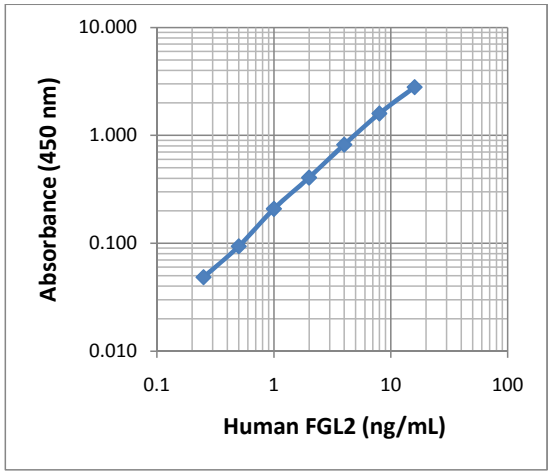
The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate 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 serum/plasma samples have been diluted prior to the assay, their measured concentrations must be multiplied by the dilution factor.

If cell culture supernatant 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 fibrinogen at up to 1 mg/mL and mouse FGL2 at up to 320 ng/mL. There was no detectable cross-reactivity when the following recombinant cytokines/chemokines were tested at up to 50 ng/mL.

Mouse	IL-1β, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, IL-17B, IL-22, IL-33, FGF-basic, GM-CSF, IFN-γ, MCP-1, SCF, TNF-α
Human	IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12/ IL-23 (p40), IL-13, IL-15, IL-22, IL-17A/F, IL-17E, IL-17F, IL-22, IL-32α, IL-33, FGF-basic, IFN-γ, TNF-α, TSLP
Rat	IL-2, TNF-α

Sensitivity: The minimum detectable concentration of FGL2 is 45 pg/mL from cell culture supernatant and 100 pg/mL from serum and plasma samples.

Recovery: FGL2 at concentrations of 16 ng/mL, 4 ng/mL, and 1 ng/mL were spiked into five human serum samples, and then analyzed with the LEGEND MAX™ Human FGL2 ELISA Kit. On average, 105.8 % of the FGL2 was recovered from the serum samples.

Linearity: Five human serum samples with high concentrations of FGL2 were diluted with Matrix C to produce samples with values within the dynamic range and then assayed with the LEGEND MAX™ Human FGL2 ELISA Kit to determine the linearity of dilution. On average, 108.3 % of the expected FGL2 was detected from the diluted samples.

Intra-Assay Precision: Two samples with varying concentration of FGL2 were assayed with sixteen replicates of each.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (ng/mL)	7.07	1.19
Standard Deviation	0.42	0.10
% CV	5.9	8.4

Inter-Assay Precision: Two human FGL2 samples, each with sixteen replicates were assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (ng/mL)	7.50	1.02
Standard Deviation	0.65	0.11
% CV	8.7	10.8

Biological Samples:

Serum - Normal human serum samples (n = 18) were assayed for basal levels of human FGL2. The mean FGL2 value was 12.3 ± 6.5 ng/mL, with a range from 2.8 ng/mL to 26.2 ng/mL.

Plasma - Normal human plasma samples (n = 5) were assayed for basal levels of human FGL2. The mean FGL2 value was 29.99 ± 21.25 ng/mL, with a range from 10.6 ng/mL to 65 ng/mL.

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.**
8999 BioLegend Way
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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