PLGF ELISA

AL-1008-r

RUO

INTENDED USE

The PLGF enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of PLGF in serum and other biological fluids. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

Placental growth factor (PLGF) is a member of PDGF/VEGF family growth factors. PLGF, a member of VEGF family, is produced mainly by placenta and is normally found in urine and blood during pregnancy. Angiogenesis and vascular transformation are important processes in the normal development of the placenta. Abnormal angiogenesis and vascular transformation are considered one of the main reasons for preeclamptic pregnancies and intrauterine growth retardation. In pre-eclampsia, there is increased expression of soluble fms-like tyrosine kinase-1 (sFlt1) which binds to circulating PLGF. Consequently, concentrations of plasma PLGF are found to be decreased in pregnant women with pre-eclampsia.

PRINCIPLE OF THE TEST

The PLGF ELISA is a quantitative two-step sandwich type immunoassay that is designed to measure human PLGF. In the first step Calibrators, Controls and unknown samples and biotinylated antibody solution are added to antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the second incubation and washing, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the PLGF antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of PLGF in the samples and calibrators.

MATERIALS SUPPLIED

CAL-1008A–CAL-1008F PLGF Calibrator A-F

Six vials, labeled A-F containing approximate concentrations of 3-750 pg/mL PLGF in a protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators A-F with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze for multiple use.

NOTE: *The PLGF concentration in the PLGF calibrators is traceable to World Health Organization preparation code 09/272 version 2.0 Dated 18/03/2013*

CTR-1008-I & CTR-1008-II PLGF Controls

Two vials, labeled Levels I and II containing low and high PLGF concentrations in protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze for multiple use.

PLT-1008 PLGF Antibody Coated Microtitration strips

One strip holder, containing 12 strips and 96 microtitration wells with PLGF antibody immobilized to the inside wall of each well. Store at 2 to 8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

BCR-1008 PLGF Biotin Conjugate Ready-to-Use

One bottle, 8 mL each, containing anti PLGF antibody in a protein buffer with a non-mercury preservative. Store at 2 to 8°C until the expiration date.

SAR-1008 PLGF Streptavidin-Enzyme Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL each, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2 to 8°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

STP-100 Stopping Solution

One bottle, 12 mL each, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microtitration orbital plate shaker.
3. Microtitration plate washer.
4. Semi-automated/manual precision pipette to deliver 2–250 µL.
5. Repeater pipette
6. Vortex mixer.
7. Deionized water.
8. Disposable 12 x 75 mm culture tubes.
9. Tight fitting 12 x 75 mm tube racks.

WARNINGS AND PRECAUTIONS

For *in vitro diagnostic use.*

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations

WARNING: Potential Biohazardous Material

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease

Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007[1].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 as a preservative. Pro-Clean 400 and peroxide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the SDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) Serum is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Samples may be stored at 4°C if assayed within 24 hours; otherwise, samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

d) Avoid assaying lipemic, hemolyzed or icteric samples.

e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.[2]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the PLGF ELISA assay. It is the responsibility of the customer to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix different lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells accurately and efficiently to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **PLGF Calibrators A-F and Controls I & II**: Tap and reconstitute PLGF Calibrator A-F and Controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
2. **Wash Solution:** Dilute Wash Concentrate A 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

Allow all samples and reagents to reach room temperature and mix reagents thoroughly by gentle inversion before use. Calibrators, controls, and samples should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Pipet **50 µL** of the Calibrators, Controls and Unknown to the appropriate wells.
3. Add **50 µL** of the PLGF Biotin Conjugate Ready-To-Use (RTU) to each well using a repeater pipette.
4. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **60 mins** at room temperature (23 ± 2°C).
5. Aspirate and wash each well **5 times** with the wash solution **(350 µL/well)** using an automatic microplate washer.
6. Add **100 µL** of the PLGF Streptavidin Conjugate ready-to-use solution to each well using a repeater pipette.
7. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **20 mins** at room temperature (23 ± 2°C).
8. Aspirate and wash each well **5 times** with the wash solution **(350 µL/well)** using an automatic microplate washer.
9. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
10. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).
    NOTE: *Visually monitor the color development to optimize the incubation time.*
11. Add **100 µL** of the **Stopping solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm.**
    NOTE*: Zero calibrator should be programmed as "**Blank**" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at **450 nm** with background wavelength correction at **630 nm**.*

RESULTS

NOTE: *The results in this package insert were calculated by plotting the **log optical density (OD) data on the y-axis and log PLGF concentration on X-axis** using a cubic regression curve fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.*

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Optimum results can be obtained at incubation temperature of (23 ± 2°C).
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the PLGF concentrations in pg/mL along the x-axis, using a cubic regression curve fit.
4. Determine the PLGF concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding PLGF concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately further diluted with the 0 pg/mL (Cal. A) and re-assayed.
6. Any sample reading lower than the LoD should be reported as such.

LIMITATIONS

1. PLGF results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings when being interpreted for diagnostic purposes.
2. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.
3. As for any assay employing antibodies, the possibility exists for interference by heterophile antibodies in the samples. Interference from heterophile antibodies has not been evaluated for this assay.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Kit controls or other commercial controls should fall within established confidence limits.
- The confidence limits for kit controls are printed on the **calibration card**.
- A full calibration curve, along with low- and high-level controls, should be included in each assay.
- TMB chromogen solution should be colorless. Development of a blue color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean Absorbance	Conc. (pg/mL)
A1, A2	Calibrator A	0.021 (**Blank**)	0
B1, B2	B	0.028	3.4
C1, C2	C	0.079	10.1
D1, D2	D	0.281	40.0
E1, E2	E	1.011	160.0
F1, F2	F	3.114	625.0

CAUTION: *The above data must not be employed in lieu of data obtained by the user in the laboratory.*

ANALYTICAL CHARACTERISTICS

Analytical Sensitivity*:*

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 pg/mL) and calibrator B (5.5 pg/mL) is 1.07 pg/mL.

Limit of Blank (LOB):

The Limit of Blank was 0.872 pg/mL, calculated from a minimum of n=18 measurements of analyte free sample.

Limit of Detection (LoD):

The lowest amount of PLGF in a sample that can be detected with a 95% probability (n=24) is 2.1 pg/mL. The value was determined by processing five samples in the range of 1.0 to 14 pg/mL following CLSI EP17 guidelines. Eight assay runs were performed over four days with samples run in triplicates per run.

Limit of Quantification (LoQ)

The Limit of Quantification (LoQ) was 2.05 pg/mL, calculated from n=24 measurements of four samples in the range of 1.0 – 7 pg/mL.

Imprecision:

Reproducibility of the PLGF ELISA assay was determined in a study using three samples. The study included a total of 6 assays, 3 replicates each, per assay (n=18). Representative data were calculated and are presented in the following table.

Sample	Mean (pg/mL)	Within run		Between run	
		SD	CV	SD	CV
Low	12.2	0.7	5.5%	0.3	2.8%
Mid	85.0	1.8	2.2%	1.5	1.8%
High	427.0	21.8	5.1%	6.3	1.5%

Linearity:

Multiple dilutions of Calibrator F and three serum samples containing various PLGF levels were diluted with Calibrator A. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution factor (1 in X)	Expected Value in pg/mL	Observed Value in pg/mL	% Recovery	Average % Recovery
Cal F	**NEAT VALUE**	717.0			**98%**
	2	358.5	347.1	**97%**	
	4	179.3	176.6	**99%**	
	8	89.6	88.2	**98%**	
	16	44.8	44.0	**98%**	
	32	22.4	22.4	**100%**	
Sample 1	**NEAT VALUE**	350.6			**102%**
	2	175.3	176.5	**101%**	
	4	87.6	89.4	**102%**	
	8	43.8	44.7	**102%**	
	16	21.9	22.1	**101%**	
	32	11.0	11.2	**102%**	
Sample 2	**NEAT VALUE**	224.2			**89%**
	2	112.1	102.7	**92%**	
	4	56.0	49.2	**88%**	
	8	28.0	25.0	**89%**	
	16	14.0	12.3	**88%**	
	32	7.0	6.2	**89%**	
Sample 3	**NEAT VALUE**	225.6			**88%**
	2	112.8	104.2	**92%**	
	4	56.4	50.1	**89%**	
	8	28.2	24.3	**86%**	
	16	14.1	11.9	**84%**	
	32	7.1	6.1	**87%**	

Recovery:

Known amounts of PLGF antigen was added to three serum samples containing different levels of endogenous PLGF. The concentration of PLGF was determined before and after the addition of exogenous PLGF and the percent recovery was calculated.

Sample ID	Endogenous Value in pg/mL	Expected in pg/mL	Observed in pg/mL	Average %Recovery
1	76.9	108.9	102.5	100%
		140.9	147.3	
		172.9	176.8	
2	99.3	130.2	124.2	91%
		161.0	142.5	
		191.9	170.4	
3	83.5	115.2	117.1	105%
		146.9	160.0	
		178.6	187.7	

Analytical Specificity:

Cross-Reactivity

Activin A, Human Activin A, PAPP-A2, ht PAPP-A, alpha 2microglobulin, Activin AB, FSTL-3, Inhibin B, Inhibin A, when tested at twice the physiological dose were below the detection limit of the assay. The antibody pair used in the assay ***detects Goat, Rabbit, Mare, sheep, Porcine, Bovine and Equine Samples.*** **It does not detect mouse, ovine, and canine.**

Cross-Reactivity with sFlt-1

sFlt-1 at 5ng/mL and 50 ng/mL concentrations were added to purified PLGF antigen at 535.4 pg/mL and 280 pg/mL concentrations. % Cross reactivity was calculated and is listed in the table below.

Sample ID	Expected PLGF (pg/mL)	spiked sFlt-1 (pg/mL)	Observed PLGF (pg/mL)	% Recovery
Ansh PLGF Antigen	267.7	5 ng/mL	257.5	96%
		50 ng/mL	194.5	73%
Ansh PLGF Antigen	138.5	5 ng/mL	124.9	90%
		50 ng/mL	92.6	67%

Hook Effect:

High-dose hook effect was not observed up to PLGF concentrations of 2000 pg/mL.

Expected Value:

The expected PLGF concentration ranges (95% CI) for PLGF in males, females and in pregnant females in first, second and 3rd trimesters were calculated and listed in the table below.

Population	No. of Specimens	Median PLGF Conc. (pg/mL)	PLGF (pg/mL) 95% CI
Male (20 – 54 Years)	147	14.8	13.1 – 16.7
Female (21 – 55 years)	86	13.5	11.4 – 16.6
10 weeks pregnant	20	20.2	16.5 - 24.5
11 weeks pregnant	20	24.6	20.9 - 30.2
12 weeks pregnant	20	30.7	25.6 - 41.2
13 weeks pregnant	20	29.2	27.1 - 33.5
15 weeks pregnant	18	41.6	32.8 - 82.9
16 weeks pregnant	18	52.7	43.8 - 62.6
17 weeks pregnant	26	99.8	64.2 - 129.4
18 weeks pregnant	23	100.4	76.1 - 119.5
19 weeks pregnant	13	141.6	87.6 - 170.3
20 weeks pregnant	5	158.3	113.2 - 178.8
3rd Trimester	11	187.5	105.6 – 335.7

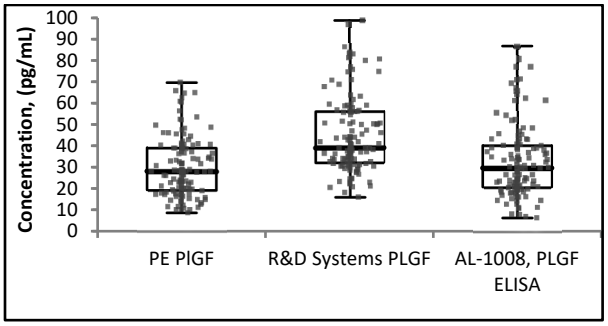
Interference:

When potential interferents (Hemoglobin, biotin, bilirubin, and intralipids) were added at least two times their physiological concentration to control sample, PLGF concentration were within ± 10% of the control as represented in the following table. This study was based on NCCLS EP-7.

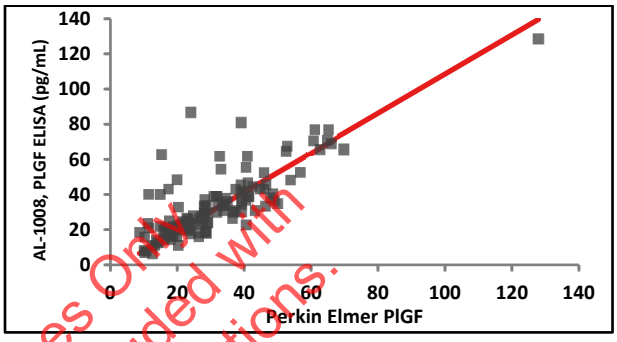
Interferent	Interferent Dose	Sample PLGF (pg/mL)	Dosed Sample PLGF (pg/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	144.5	149.4	3.4
	0.5 mg/mL	161.8	159.4	-1.5
	0.1 mg/mL	269.5	272.8	1.2
Hemoglobin	1 mg/mL	153.0	152.6	-0.3
	0.5 mg/mL	254.6	268.3	5.4
	0.1 mg/mL	280.7	274.6	-2.2
Biotin	1200 ng/mL	148.0	153.1	3.4
	600 ng/mL	165.2	161.8	-2.1
	200 ng/mL	269.4	272.7	1.2
Biotin	1200 ng/mL	158.2	157.3	-0.6
	600 ng/mL	264.6	254.0	-4.0
	200 ng/mL	274.3	277.4	1.1
Intralipids	20 mg/mL	159.4	153.8	-3.6
	10 mg/mL	164.4	159.9	-2.8
	5 mg/mL	278.9	254.1	-8.9
Intralipids	20 mg/mL	160.8	157.6	-2.0
	10 mg/mL	265.0	248.8	-6.1
	5 mg/mL	276.4	287.5	4.0
Bilirubin	0.66 mg/mL	111.4	111.6	0.2
	0.2 mg/mL	194.4	192.8	-0.8
Bilirubin	0.66 mg/mL	150.9	146.4	-3.0
	0.2 mg/mL	258.1	246.1	-4.7

Method Comparison:

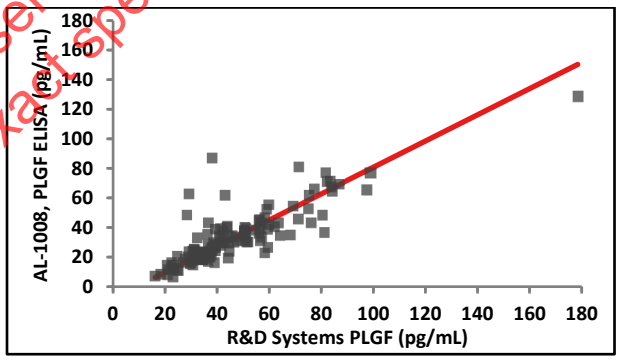
129 Serum samples with known PlGF concentration were compared between Ansh Labs PLGF (AL-1008) ELISA, PE PLGF and R&D Systems PLGF ELISAs. The observed mean concentrations were 33.61, 30.92, and 46.21 respectively.



A regression analysis was performed between Perkin Elmer and Ansh Labs PLGF observed concentrations. Ansh Labs PLGF Conc. =1.11 (Perkin Elmer)known PLGF Value -2.72 pg/mL, (R=0.802)

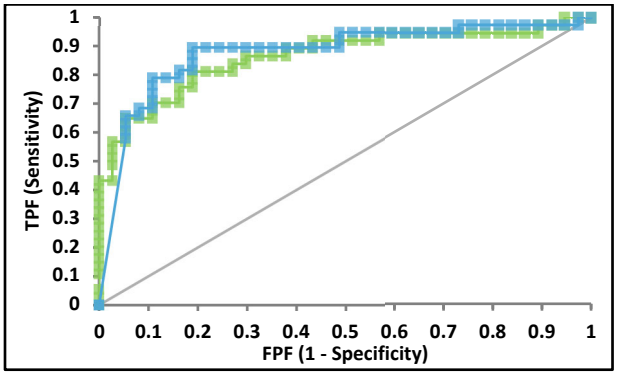


A regression analysis was performed between R&D system and Ansh PLGF values. Ansh Labs PLGF Conc. =0.886 (R&D Systems) PLGF Value -7.74 pg/mL, (R=0.840)



Method Comparison

79 samples with known PLGF values from R&D System were compared with Ansh PLGF (AL-1008) ELISA. Diagnostic performance (ROC Curve) analysis of the results was done using Analyse-it for Microsoft Excel.



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This assay is intended for in-vitro diagnostic use only. Not for sale in U.S.A

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