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Mouse PLGF SimpleStep ELISA® Kit

For the quantitative measurement of PLGF in mouse serum, plasma (citrate), and cell culture supernatant samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

PLGF *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of PLGF protein in mouse serum, plasma (citrate), and cell culture supernatant samples

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Placental growth factor (PLGF) is an angiogenic factor that is variably glycosylated and secreted. The PLGF protein is a 55-60 kDa disulfide linked homodimer and it belongs to the small family of PDGF/VEGF-related angiogenic growth factors, all of which contain 8 conserved cysteines. Due to alternative splicing, there are three human PLGF mRNAs encoding human PLGF-1, -2, and -3 isoform precursors. There is a unique 21 aa insert at the C-terminus of the PLGF-2 isoform and this isoform is the heparin binding form of PLGF. In mouse tissues there is currently only one identified PLGF mRNA form encoding the equivalent of human PLGF-2. Mouse PLGF is a 158 aa polypeptide that contains an 18 aa signal sequence and a 140 aa mature segment. Human and rat PLGF share 66% and 91% aa sequence identity, respectively, to mouse PLGF.

Homodimeric mouse PLGF binds the tyrosine kinase receptor VEGF R1/Flt-1. This receptor is a 180 kDa type I transmembrane glycoprotein with high affinity for PLGF. In addition to forming homodimers, PLGF also forms heterodimers with VEGF-A isoforms,

forming bioactive molecules. When heterodimerized with VEGF, PLGF will activate VEGF R2.

A limited number of functions have been directly attributed to PLGF. It is known to induce monocyte activation and to provide a stimulus for monocyte migration. PLGF also upregulates VEGF production in PBMCs and induces tissue factor secretion in both monocytes and HUVECs. Furthermore, PLGF is also reported to serve as a growth and chemotactic factor for endothelial cells. Finally, PLGF is known to be expressed by trophoblasts and may protect against growth factor withdrawal-induced trophoblast apoptosis.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Mouse PLGF Capture Antibody 10X	600 µL	+4°C
Mouse PLGF Detector Antibody 10X	600 µL	+4°C
Mouse PLGF Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BI	6 mL	+4°C
Sample Diluent 25BS	20 mL	+4°C
Sample Diluent 75BP	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Sample Diluents 25BS and 75BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

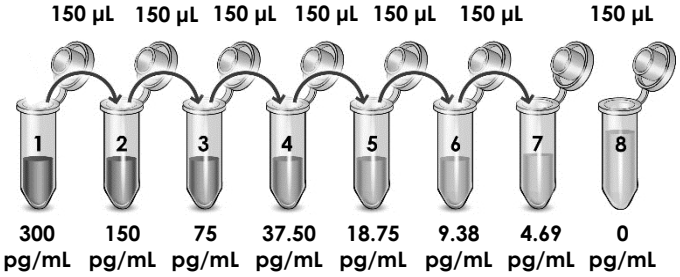
10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the PLGF standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the PLGF standard by adding 1 mL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 300 pg/mL **Standard #1** Solution.
For **serum sample measurements**, reconstitute the PLGF standard by adding Sample Diluent 25BS.
For **plasma sample measurements**, reconstitute the PLGF standard by adding Sample Diluent 75BP.
For **cell culture supernatant sample measurements**, reconstitute the PLGF standard by adding Sample Diluent NS.

10.1.1 Label eight tubes, Standards 2– 8 and add 150 µL of appropriate diluent (see step 10.1) into each tube.

10.1.3 Use the Standard #1 to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	1: 32 – 1: 2
Plasma – Citrate	1: 32 – 1: 2
Mouse Lung Supernatant	1: 320 – 1: 5
Stimulated L292 Supernatant	6.25 – 100%

11.1 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1: 2 into Sample Diluent 25BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.2 Plasma:

Collect plasma using citrate. Plasma collected using EDTA or heparin has not been tested with this kit. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1: 2 into Sample Diluent 75BP and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples as needed into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed

time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

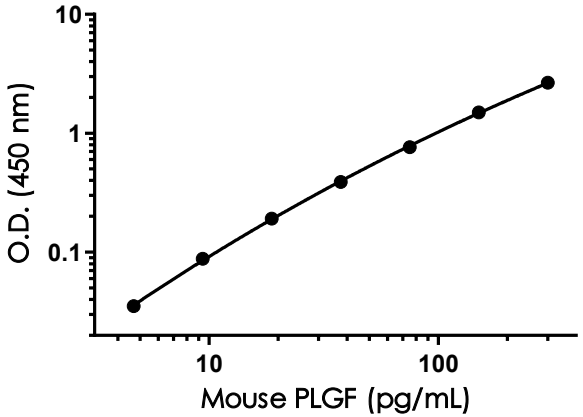
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

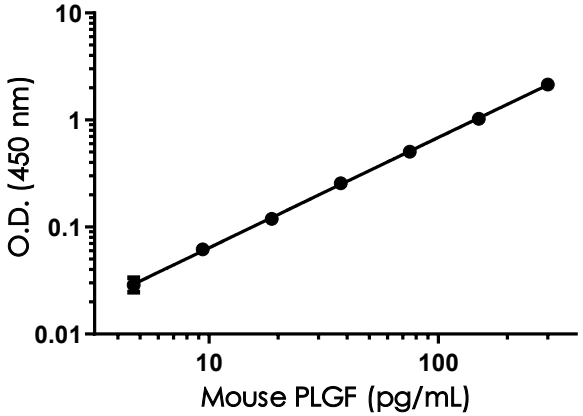
15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



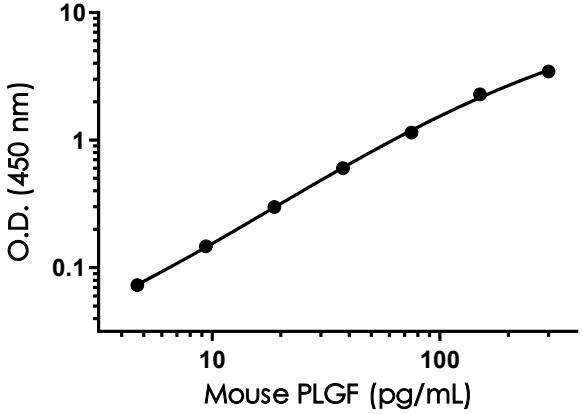
Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.070	0.077	0.074
4.69	0.120	0.116	0.118
9.38	0.175	0.167	0.171
18.75	0.267	0.282	0.275
37.50	0.460	0.486	0.473
75	0.824	0.866	0.845
150	1.536	1.627	1.582
300	2.615	2.879	2.750

Figure 1. Example of mouse PLGF standard curve in Sample Diluent 25BS. The PLGF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.055	0.058	0.056
4.69	0.088	0.094	0.091
9.38	0.121	0.126	0.124
18.75	0.175	0.187	0.181
37.50	0.307	0.330	0.318
75	0.545	0.591	0.568
150	1.020	1.160	1.090
300	2.130	2.296	2.213

Figure 2. Example of mouse PLGF standard curve in Sample Diluent 75BP. The PLGF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.074	0.071	0.072
4.69	0.146	0.153	0.149
9.38	0.218	0.228	0.223
18.75	0.369	0.381	0.375
37.50	0.672	0.688	0.680
75	1.149	1.303	1.226
150	2.297	2.434	2.365
300	3.557	3.518	3.537

Figure 3. Example of mouse PLGF standard curve in Sample Diluent NS. The PLGF standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent 25BS	24	0.526 pg/mL
Sample Diluent 75BP	24	1.559 pg/mL
Sample Diluent NS	24	0.829 pg/mL

RECOVERY –

Three concentrations of mouse PLGF were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Mouse Serum	104	101 – 107
50% Mouse Plasma - Citrate	111	101 – 119
100% Cell Culture Media	98	96 – 101

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native PLGF was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 25BS.

Dilution Factor	Interpolated value	50% Mouse Serum
Undiluted	pg/mL	51
	% Expected value	100
2	pg/mL	26
	% Expected value	100
4	pg/mL	13
	% Expected value	99
8	pg/mL	6
	% Expected value	101
16	pg/mL	4
	% Expected value	109

Native PLGF was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 75BP.

Dilution Factor	Interpolated value	50% Mouse Plasma (Citrate)
Undiluted	pg/mL	45
	% Expected value	100
2	pg/mL	21
	% Expected value	94
4	pg/mL	10
	% Expected value	91
8	pg/mL	5
	% Expected value	85
16	pg/mL	3
	% Expected value	91

Native PLGF was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	5% Mouse Lung Supernatant	100% Stimulated Mouse L929 Supernatant
Undiluted	pg/mL	328	179
	% Expected value	100	100
2	pg/mL	182	83
	% Expected value	111	92
4	pg/mL	78	39
	% Expected value	95	86
8	pg/mL	35	18
	% Expected value	85	79
16	pg/mL	16	9
	% Expected value	80	79

Recombinant PLGF was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS

Dilution Factor	Interpolated value	100% Cell Culture Media
Undiluted	pg/mL	153
	% Expected value	100
2	pg/mL	74
	% Expected value	97
4	pg/mL	37
	% Expected value	96
8	pg/mL	19
	% Expected value	100
16	pg/mL	10
	% Expected value	109

PRECISION –

Mean coefficient of variations of interpolated values of PLGF from three concentrations of mouse serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	4.7	6.5

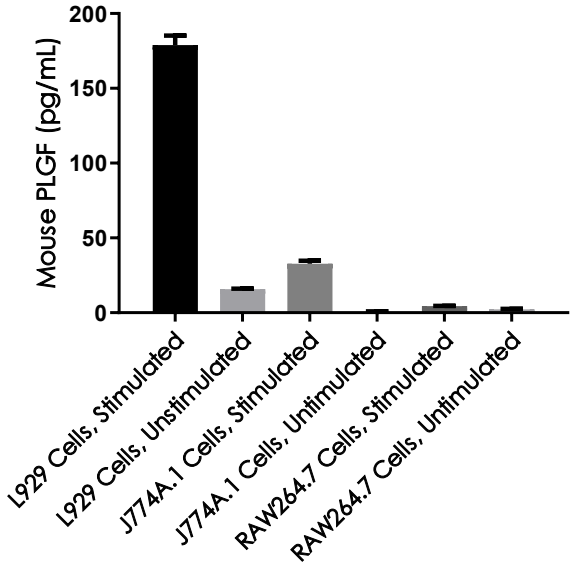


Figure 4. PLGF Mouse expression is shown for cultured media from three Mouse cell lines. L929, J774A.1 and RAW264.7 cells were cultured in HGDMEM with 10% fetal calf serum or Horse serum, 2mM L-glutamine and 100 µg/mL Kanamycin. L929 and J774A.1 cells were treated for 72 hours in the presence and absence of 1.5% PHA and 10 ng/mL of PMA. RAW264.7 cells were starved for 24 hours and treated in the presence and absence of 5 µg/mL of LPS. Samples were loaded on the plate undiluted and the concentrations of PLGF were interpolated from a calibration curve diluted in NS. The mean PLGF concentration was determined to be 178.9 pg/mL in stimulated L929 cells, 15.8 pg/mL in unstimulated L929 cells, 32.8 pg/mL in stimulated J774A.1 cells, 0.4 pg/mL in unstimulated J774A.1 cells, 4.4 pg/mL in stimulated RAW264.7 cells, and 2.3 pg/mL in unstimulated RAW264.7 cells.

17. Assay Specificity

This kit recognizes both native and recombinant mouse PLGF protein in serum, plasma (citrate), and cell culture supernatant samples only.

Plasma samples collected with heparin and EDTA have not been tested with this kit.

CROSS REACTIVITY

The recombinant proteins listed below were prepared at 50 ng/mL in Sample Diluent NS and assayed for cross reactivity. No significant cross reactivity was observed, with a mean O.D. deviation from background of 0.0043.

Mouse recombinant proteins:

VEGF	CXCL4	IL-2
VEGF R1	TNF-alpha	SCF
VEGF R3	IL-12p40	

Human recombinant proteins:

VEGF	VEGF R1

INTERFERENCE

Recombinant PLGF control was assayed at 75 pg/mL in the presence and absence of several different concentrations of mouse VEGF recombinant protein to determine interference. The expected % recovery of mouse PLGF is shown below.

VEGF mouse (pg/mL)	Expected % Recovery
750	96.8
7,500	94
50,000	95

Recombinant PLGF control was assayed at 75 pg/mL and 300 pg/mL in the presence and absence of several different concentrations of mouse VEGF R1 recombinant protein to determine interference. The expected % recovery of mouse PLGF is shown below. Similar results were obtained in the presence and absence of recombinant Human VEGF R1.

	Expected % Recovery	
VEGF R1 (pg/mL)	75 pg/mL PLGF	300 pg/mL PLGF
150	78.7	97.4
750	30.1	89.3
1,500	16.6	70.6

Similar results were obtained in the presence and absence of recombinant Human VEGF R1.

Recombinant PLGF control was assayed at 75 pg/mL in the presence and absence of 50 ng/mL of the remaining recombinant proteins listed previously to determine interference. Recovery of PLGF was observed on average at 101.9% with a standard deviation of 7.3%.

18. Species Reactivity

This kit recognizes mouse PLGF protein.

Other species reactivity was determined by measuring a 1:2 dilution of serum samples of various species, interpolating the PLGF protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the PLGF protein concentration in mouse serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Human
- Hamster
- Guinea Pig
- Rabbit
- Dog
- Goat
- Pig
- Cow
- Chicken

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)