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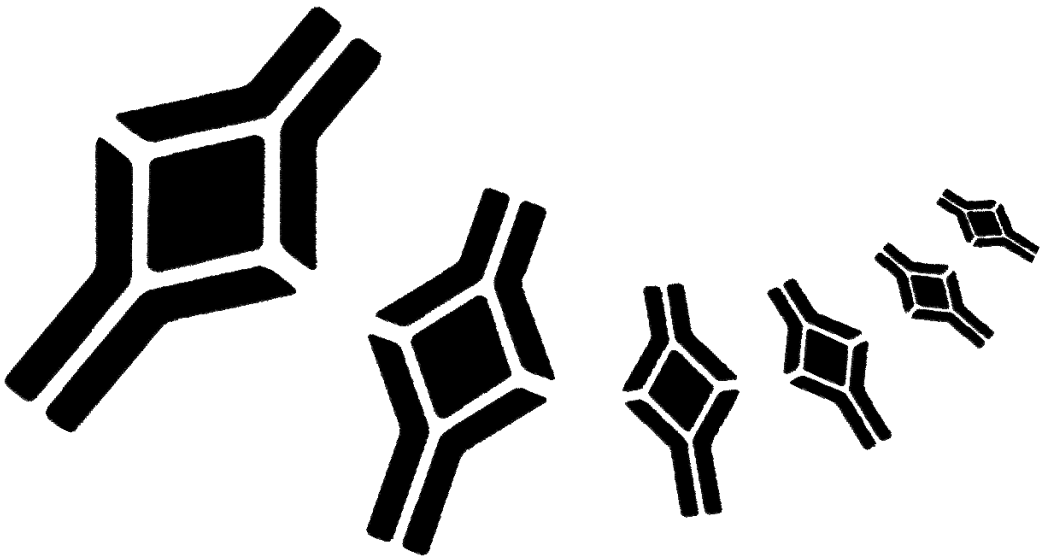


Human sPSGL-1 ELISA

Product Data Sheet

Cat. No.: RBMS255R

For Research Use Only

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína, a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1 INTENDED USE

The sPSGL-1 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human soluble P-selectin Glycoprotein Ligand-1 (PSGL-1) in cell culture supernatants, human serum, plasma, or other body fluids. **The sPSGL-1 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2 SUMMARY

The family of selectins consists of three structurally and functionally related molecules. L-selectin is constitutively expressed on neutrophils, P-selectin is found on platelets and is stored in Weibel-Palade bodies from where it is transported to the cell surface upon endothelial activation. E-selectin is expressed on endothelial cells. Due to a common structural element, the amino-terminal lectin-like domain, the selectins are able to bind to carbohydrate ligands. Different putative ligand structures have been identified for which the selectins show high affinity. These structures include oligosaccharides, phophorylated saccharides, sulfopolysaccharides and lipids.

It was shown that glycoproteins represent the biological relevant ligands for selectins. While the ligands for E- and L-selectins with primary binding activity have not been identified so far, the functionally most important ligand for P-selectin has been identified. The mucin-like glycoprotein PSGL-1 (P-selectin Glycoprotein Ligand-1) has been cloned and sequenced.

PSGL-1 has been shown to be a transmembrane protein which forms homodimers via disulfide bridges of two 120 kDa chains.

PSGL-1 is expressed on cells of myeloid, lymphoid and dendritic lineage. The binding of P-selectin is regulated by different degrees and forms of glycosylation. An interaction of L-selectin with PSGL-1 in the process of neutrophil aggregation has been shown. However, PSGL-1 does not seem to be the primary ligand for L-selectin.

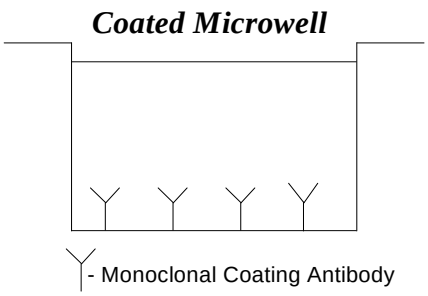
Presently it is not known which cells, apart from leukocytes, express PSGL-1 and what role PSGL-1 plays on these cells. The metastic potential of the majority of cells which bind to P-selectin is however in close correlation with the functional expression of PSGL-1 on these cells.

The regulation of PSGL-1 is not yet well described. Glycosyltransferases sure play an important role in activation. The deactivation of PSGL-1 is so far unclear. The cleavage of the protein from the cell surface is one mechanism involved in the deactivation process.

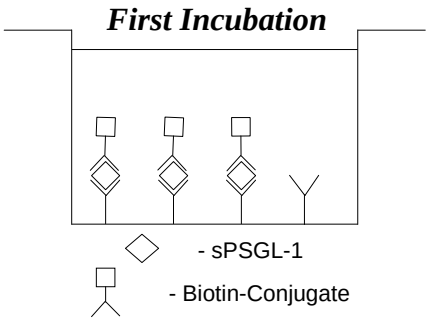
Following this shedding, a soluble form of PSGL-1 is detectable in the circulation. This soluble isoform of PSGL-1 is still capable of binding to P-selectin, thus representing a competitor for cellular PSGL-1 through which regulation in many physiological and pathological processes can take place.

3 PRINCIPLES OF THE TEST

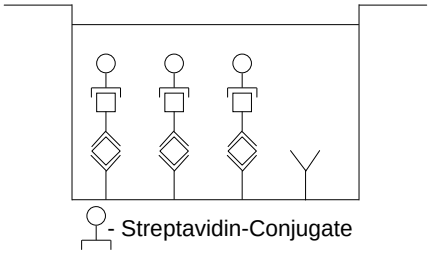
An anti-sPSGL-1 monoclonal coating antibody is adsorbed onto microwells.

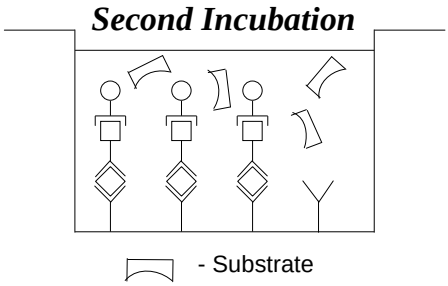


sPSGL-1 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-sPSGL-1 antibody is added and binds to sPSGL-1 captured by the first antibody.

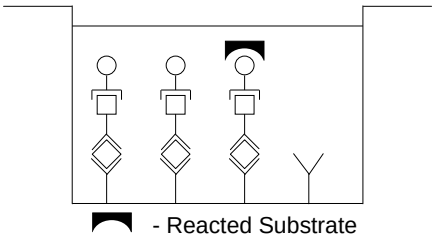


Following incubation unbound biotin-conjugated anti-sPSGL-1 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-sPSGL-1: Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.





A coloured product is formed in proportion to the amount of sPSGL-1 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from six sPSGL-1 standard dilutions and sPSGL-1 sample concentration determined.



4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with Monoclonal Antibody (murine) to human sPSGL-1

1 vial (100 µl) **Biotin-Conjugate** anti-sPSGL-1 monoclonal (murine) antibody

2 vials **sPSGL-1 Standard,** lyophilized, 100 U/ml upon reconstitution

1 vial (150µl) **Streptavidin-HRP**

1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)

1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA)

1 vial (50 ml) **Sample Diluent** (protein matrix)

1 vial (15 ml) **Substrate Solution**

1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml) **Blue-Dye**

1 vial (0.4 ml) **Green-Dye**

1 vial (0.4 ml) **Red-Dye**

4 adhesive **Plate Covers**

Reagent Labels

5 STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 SPECIMEN COLLECTION

Cell culture supernatants, human serum, plasma, or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive sPSGL-1. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to 13. E, and F.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes

- 10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips

- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips

- Multichannel micropipette reservoir

- Beakers, flasks, cylinders necessary for preparation of reagents

- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)

- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

- Glass-distilled or deionized water

- Statistical calculator with program to perform linear regression analysis.

8 PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice

- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

- Do not mix or substitute reagents with those from other lots or other sources.

- Do not use kit reagents beyond expiration date on label.

- Do not expose kit reagents to strong light during storage or incubation.

- Do not pipette by mouth.

- Do not eat or smoke in areas where kit reagents or samples are handled.

- Avoid contact of skin or mucous membranes with kit reagents or specimens.

- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.

- Avoid contact of substrate solution with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.

- Exposure to acids will inactivate the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.

- Substrate solution must be at room temperature prior to use.

- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 PREPARATION OF REAGENTS

Prepare Wash Buffer (reagent A) and Assay Buffer (reagent B) before starting with the test procedure.

A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.
Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.
Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

B. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the Biotin-Conjugate with **Assay Buffer** (reagent B) in a clean plastic tube.
The Biotin-Conjugate may be prepared as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Preparation of Streptavidin-HRP

Make a 1:200 dilution of the concentrated Streptavidin-HRP solution as needed according to the following table:

Number of Strips	Streptavidin-HRP (µl)	Assay Buffer (ml)
1 - 6	30	6
1 - 12	60	12

E. Preparation of sPSGL-1 Standard

Reconstitute **Standard** by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard. Shake gently to ensure complete solubilisation. Discard reconstituted standard not needed.

F. Addition of colour-giving reagents: Blue-Dye, Green-Dye, Red-Dye

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.
Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye, Red-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 ml Sample Diluent	20 µl ***Blue-Dye***
12 ml Sample Diluent	48 µl ***Blue-Dye***
24 ml Sample Diluent	96 µl ***Blue-Dye***
48 ml Sample Diluent	192 µl ***Blue-Dye***

2. Biotin-Conjugate: Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of Biotin-conjugate.

3 ml Assay Buffer	30 µl ***Green-Dye***
6 ml Assay Buffer	60 µl ***Green-Dye***
12 ml Assay Buffer	120 µl ***Green-Dye***

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP; add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

6 ml Assay Buffer	24 µl ***Red-Dye***
12 ml Assay Buffer	48 µl ***Red-Dye***

10 TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

b. Dilute serum or plasma samples 1:20 with Sample Diluent according to the following dilution scheme:
Add 15 µl Sample to 285 µl Sample Diluent. Mix thoroughly.
For cell culture supernatants optimal dilutions have to be determined.

c. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (murine) to human sPSGL-1 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

d. Wash the microwell strips twice with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

e. Add 100 µl of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (refer to preparation of reagents, 9.E.) **sPSGL-1 Standard**, in duplicate, into well A1 and A2 (see Figure 1 and 2). Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure four times, creating two rows of sPSGL-1 standard dilutions ranging from 50 to 1.6 U/ml. Discard 100 µl of the contents from the last microwells (F1, F2) used.

Figure 1. Preparation of sPSGL-1 standard dilutions:

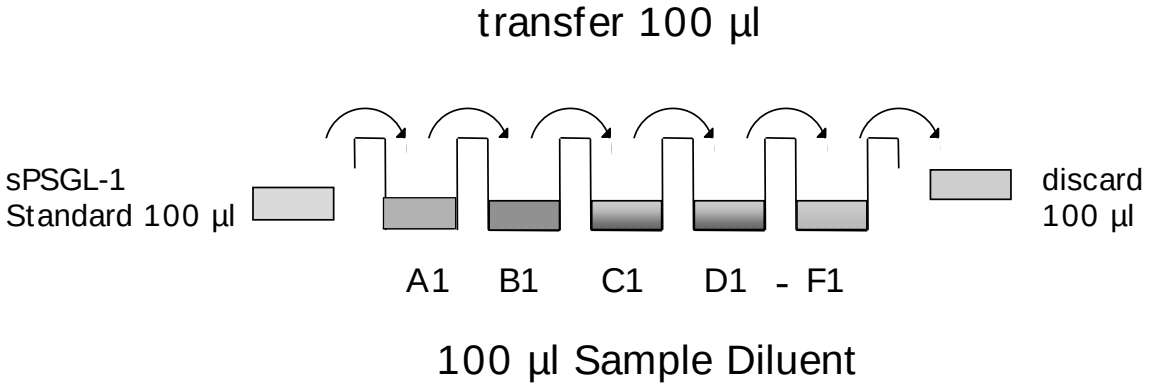


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (50 U/ml)	Standard 1 (50 U/ml)	Sample 2	Sample 2
B	Standard 2 (25 U/ml)	Standard 2 (25 U/ml)	Sample 3	Sample 3
C	Standard 3 (12.5 U/ml)	Standard 3 (12.5 U/ml)	Sample 4	Sample 4
D	Standard 4 (6.3 U/ml)	Standard 4 (6.3 U/ml)	Sample 5	Sample 5
E	Standard 5 (3.2 U/ml)	Standard 5 (3.2 U/ml)	Sample 6	Sample 6
F	Standard 6 (1.6 U/ml)	Standard 6 (1.6 U/ml)	Sample 7	Sample 7
G	Blank	Blank	Sample 8	Sample 8
H	Sample 1	Sample 1	Sample 9	Sample 9

f. Add 100 µl of **Sample Diluent,** in duplicate, to the blankwells.

g. Add 100 µl of each prediluted **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate** (refer to preparation of reagents).

i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 2 hours, if available on a rotator set at 100 rpm.

k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point d. of the test protocol. Proceed immediately to the next step.

l. Prepare **Streptavidin-HRP** (refer to preparation of reagents).

m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Cover** and incubate at room temperature (18º to 25ºC) for 1 hour, if available on a rotator set at 100 rpm.

o. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point d. of the test protocol. Proceed immediately to the next step.

p. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.

q. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point r. of this protocol) before positive wells are no longer properly recordable.
It is recommended to add the stop solution when the highest standard has developed a dark blue colour.
Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.

r. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

s. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the sPSGL-1 standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the sPSGL-1 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating sPSGL-1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding sPSGL-1 concentration.

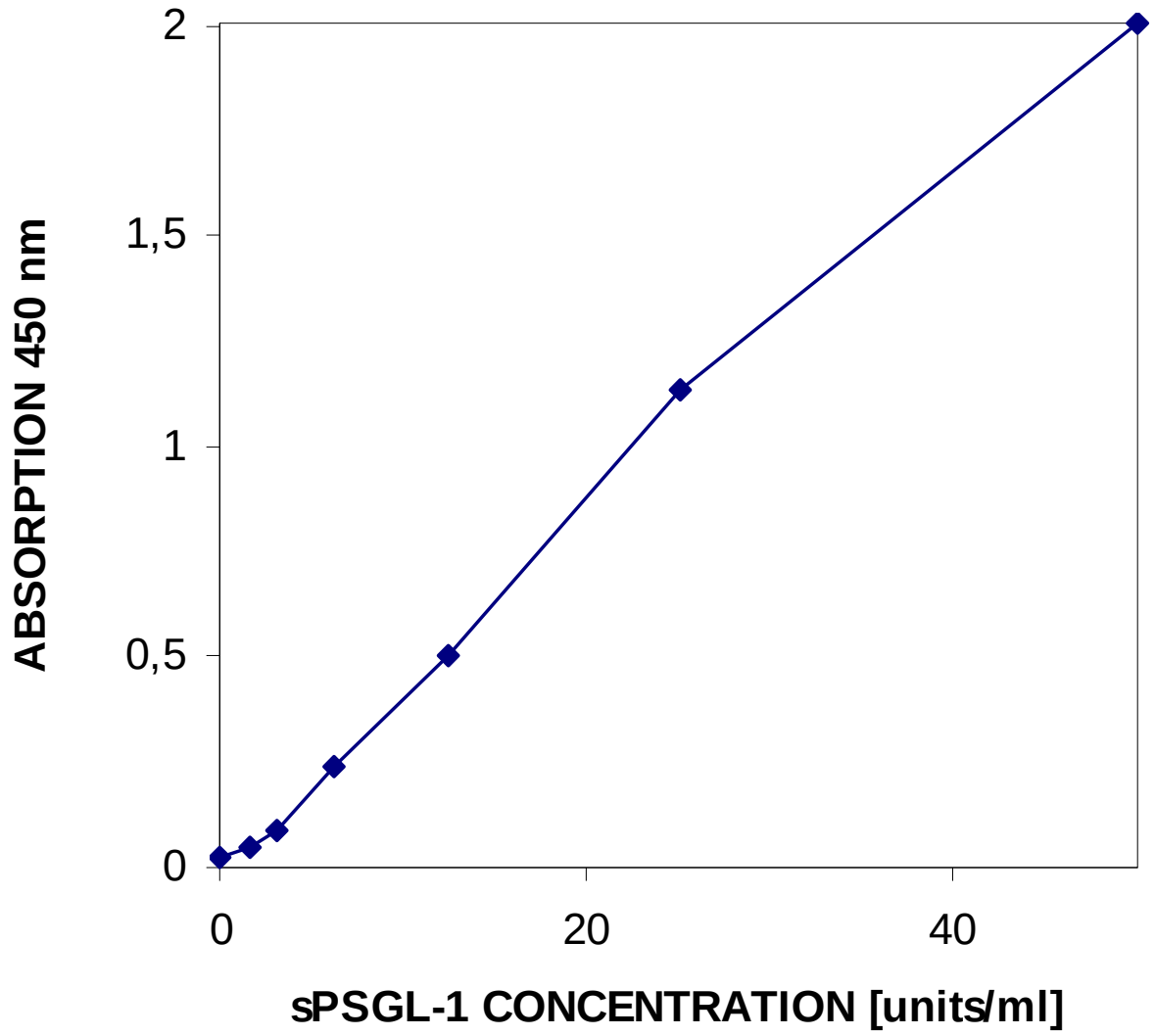
- **For samples which have been diluted according to the instructions given in this manual 1:20, the concentration has to be multiplied by the dilution factor (x20).**

  Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sPSGL-1 levels. Such samples require further dilution of 1:40 - 1:80 with Sample Diluent in order to precisely quantitate the actual sPSGL-1 level.

- It is suggested that each testing facility establishes a control sample of known sPSGL-1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for sPSGL-1 ELISA. sPSGL-1 was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations.
Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the sPSGL-1 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	sPSGL-1 Concentration (U/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	50	2.041	2.006	2.5
	50	1.970		
2	25	1.107	1.138	3.8
	25	1.168		
3	12.5	0.502	0.504	0.6
	12.5	0.506		
4	6.3	0.223	0.237	8.1
	6.3	0.225		
5	3.2	0.090	0.089	1.6
	3.2	0.088		
6	1.6	0.048	0.05	4.3
	1.6	0.051		
Blank	0	0.026	0.024	
	0	0.022		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. temperature effects). Furthermore shelf life of the kit may effect enzymatic activity and thus colour intensity. Values measured are still valid.

12 LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

- The use of immunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the Sample Diluent.

13 PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of sPSGL-1 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.99 U/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sPSGL-1. Two standard curves were run on each plate. Data below show the mean sPSGL-1 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 3.2%.

Positive Sample	Experiment	sPSGL-1 Concentration (U/ml)	Coefficient of Variation (%)
1	1	55.2	1.6
	2	54.5	5.7
	3	62.5	1.4
2	1	45.8	4.4
	2	48.8	1.4
	3	50.7	2.8
3	1	83.6	6.7
	2	94.3	2.7
	3	106.7	0.7
4	1	29.2	9.0
	2	31.5	3.3
	3	33.4	2.8
5	1	58.6	7.3
	2	61.4	1.9
	3	70.2	1.1
6	1	38.1	8.9
	2	42.2	3.3
	3	43.3	0.9
7	1	61.5	2.3
	2	63.9	1.5
	3	64.7	0.7
8	1	66.8	0.8
	2	65.1	4.7
	3	68.2	1.6

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sPSGL-1. Two standard curves were run on each plate. Data below show the mean sPSGL-1 concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 6.6 %.

Sample	sPSGL-1 Concentration (U/ml)	Coefficient of Variation (%)
1	57.4	7.7
2	48.4	5.1
3	94.8	12.2
4	31.3	6.8
5	63.4	9.5
6	41.2	6.6
7	63.4	2.6
8	66.7	2.3

C. Spiking Recovery

The spiking recovery was evaluated by spiking four levels of sPSGL-1 into four normal human sera. Recoveries were determined in three independent experiments with 4 replicates each. The amount of endogenous sPSGL-1 in unspiked serum was substracted from the spike values. Recoveries ranged from 83 to 104% with an overall mean recovery of 94%.

D. Dilution Linearity

Four serum samples with different levels of sPSGL-1 were assayed at four serial two-fold dilutions with 4 replicates each. In the table below the per cent recovery of expected values is listed. Recoveries ranged from 88% to 100% with an overall mean recovery of
95.3 %.

Sample	Dilution	sPSGL-1 Concentration (U/ml) Expected Value	Observed Value	% Recovery of Exp. Value
1	1:20	-	89.8	-
	1:40	44.9	41.2	92
	1:80	22.4	19.7	88
	1:160	11.2	10.2	91
2	1:20	-	117	-
	1:40	58.5	56	96
	1:80	29.3	29	98
	1:160	14.6	15	100
3	1:20	-	97.1	-
	1:40	48.6	47.8	99
	1:80	24.3	23.3	96
	1:160	12.1	11.3	93
4	1:20	-	83.1	-
	1:40	41.5	40.1	97
	1:80	20.8	20.1	97
	1:160	10.4	10.3	99

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed up to 5 times, and sPSGL-1 levels determined. There was no significant loss of sPSGL-1 by freezing and thawing up to 5 times.

b. Storage Stability

Aliquots of a serum sample (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the sPSGL-1 level determined after 24 h. There was no significant loss of sPSGL-1 immunoreactivity during storage under above conditions.

F. Comparison of Serum and Plasma

From eight individuals, serum as well as EDTA and citrate, and heparin plasma obtained at the same time point were evaluated. sPSGL-1 concentrations were not significantly different and therefore all these body fluids are suitable for the assay. It is nevertheless highly recommended to assure the uniformity of blood preparations.

G. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a serum sample. There was no detectable cross reactivity.

H. Expected Values

A panel of 22 sera from apparently healthy blood donors (males and females) was tested for sPSGL-1. The serum levels ranged from 329.8 U/ml to 762.5 U/ml with an average value of 451.0 U/ml. The normal levels measured may however vary with the sample collective used.

14 REFERENCES

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15 REAGENT PREPARATION SUMMARY

A. Wash Buffer Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

B. Assay Buffer

Number of Strips	Assay Buffer Concentr. (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Biotin-Conjugate Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Streptavidin-HRP

Number of Strips	Streptavidin-HRP (µl)	Assay Buffer (ml)
1 - 6	30	6.0
1 - 12	60	12.0

E. Standard Reconstitute **Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial.

16 TEST PROTOCOL SUMMARY

- Make **1:20 dilutions** of samples in Sample Diluent
- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells.
- Pipette 100 µl reconstituted **sPSGL-1 Standard** into the first wells and create standard dilutions ranging from 50 to 1.6 U/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 100 µl prediluted **Sample**, in duplicate, to designated wells
- Prepare **Biotin-Conjugate**
- Add 50 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C)
- Prepare **Streptavidin-HRP**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature (18º to 25ºC)
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 10 to 20 minutes at room temperature (18°to 25°C).
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sPSGL-1 levels. Such samples require further dilution of 1:40 - 1:80 with Sample Diluent in order to precisely quantitate the actual sPSGL-1 level.

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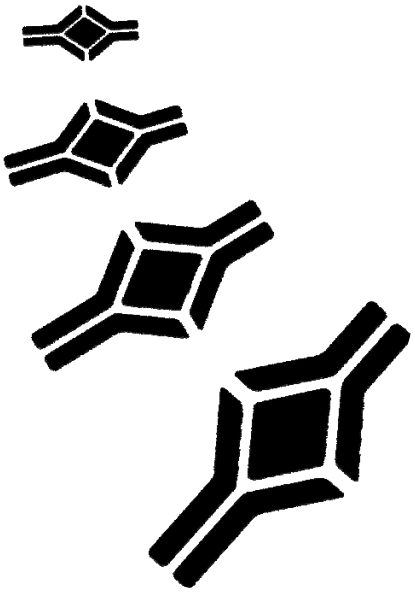

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