Quantikine® ELISA

Human PSG-1 Immunoassay

Catalog Number DPSG10

For the quantitative determination of human Pregnancy-Specific beta-1-Glycoprotein 1 (PSG-1) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
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DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
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China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
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INTRODUCTION

Pregnancy-specific beta-1-glycoprotein 1 (PSG-1), also called SP1, PSβG1 or B1G1 and designated CD66f, is a secreted glycoprotein of the human PSG family within the CEA (carcinoembryonic antigen) superfamily (1-4). Mature human PSG-1 contains a V-type Ig-like domain that is important for adhesion (N1), and three C2-type Ig-like domains (A1, A2 and B2). Human and mouse PSG family members likely share common functions, although sequence identity between rodent and human PSG proteins is limited and is mainly within the N1 domain (4-6). There is no specific mouse ortholog for PSG-1. PSG-1 shares 84-91% amino acid (aa) sequence identity with the most similar human family members, PSG-3, -4, -6, -7, and -8. Many early studies were performed using mixtures of purified human PSGs, and antibodies that recognize multiple human PSG family members (1, 2, 7-10). However, PSG-1 is by far the most highly expressed family member in the maternal plasma during pregnancy (10).

PSG-1 is produced by syncytiotrophoblast cells beginning with their differentiation from villous cytotrophoblast cells (3, 11). It becomes detectable in the maternal plasma as early as 2-3 weeks after fertilization, and increases as pregnancy progresses (12). Lower than average plasma PSG-1 in the first or early second trimester has been correlated with fetal growth restriction, low birth weight, or pre-term delivery (13-16). Expression of PSGs has also been detected in choriocarcinomas, hydatidiform moles, ovarian adenocarcinomas, and breast tumors (2, 9, 17). Fibroblast production of PSG-1 mRNA, intracellular protein, or very low amounts of secreted protein has also been reported (18).

Although mouse PSG-17 and PSG-19 are ligands for CD9, human PSG-1 is not and instead mediates cell adhesion via heparan and chondroitin sulfate proteoglycans such as Syndecans and Glypican-1 (19). PSG-1 upregulates monocyte, macrophage and trophoblast cell production of TGF-β1, which enhances VEGF production and endothelial tube formation *in vitro* and indicates a possible role in placental vascular morphogenesis (5, 7, 19). It also promotes monocyte alternate activation, and upregulates monocyte production of IL-10 and IL-6 (5, 21, 22). In *in vitro* testing of T-cells, it inhibits the mixed lymphocyte reaction and promotes a Th2-type immune environment (5, 8, 22). PSG-1 is thus implicated in creation of an anti-inflammatory uterine environment (5, 20-22).

The Quantikine Human PSG-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human PSG-1 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human PSG-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human PSG-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human PSG-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PSG-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PSG-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for PSG-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PSG-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by soluble recptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
PSG-1 Microplate	894217	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against PSG-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
PSG-1 Conjugate	894218	21 mL of a monoclonal antibody against PSG-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
PSG-1 Standard	894219	1000 ng of recombinant human PSG-1 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-75	895811	11 mL of buffered animal serum with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD6F	895018	21 mL of buffered animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human PSG-1 Controls (optional; available from R&D Systems).

PRECAUTIONS

Calibrator Diluent RD6F contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

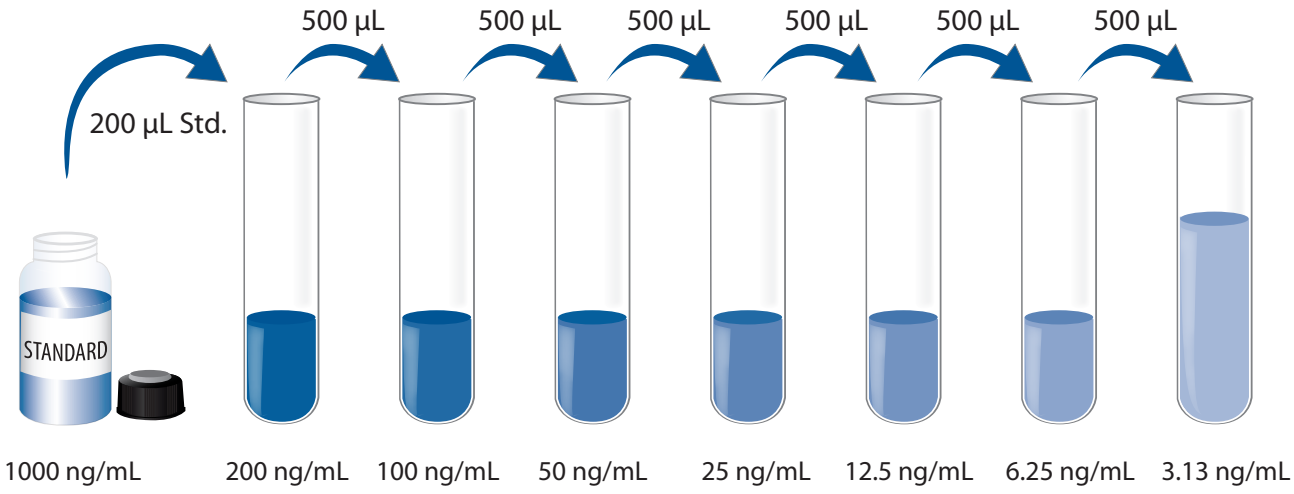
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6F (1:5) - Dilute 4 mL of Calibrator Diluent RD6F into 16 mL of deionized or distilled water to yield 20 mL of Calibrator Diluent RD6F (1:5).

PSG-1 Standard - Reconstitute the PSG-1 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 1000 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 800 µL of Calibrator Diluent RD6F (1:5) into the 200 ng/mL tube. Pipette 500 µL Calibrator Diluent RD6F (1:5) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard. Calibrator Diluent RD6F (1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-75 to each well. *May contain a precipitate. Mix well before and during use.*
4. Add 50 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of PSG-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

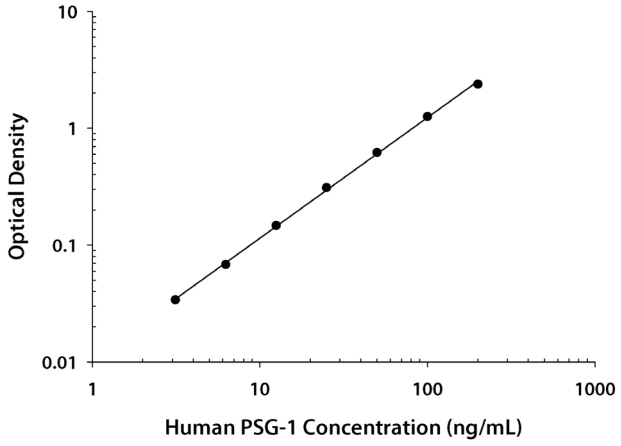
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the PSG-1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.009	0.009	—
3.13	0.042 0.044	0.043	0.034
6.25	0.077 0.077	0.077	0.068
12.5	0.154 0.157	0.156	0.147
25	0.315 0.323	0.319	0.310
50	0.626 0.633	0.630	0.621
100	1.250 1.284	1.267	1.258
200	2.372 2.415	2.394	2.385

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	18.7	59.4	118	20.0	62.4	121
Standard deviation	0.36	0.79	1.7	1.2	2.4	4.9
CV (%)	1.9	1.3	1.4	6.0	3.8	4.0

RECOVERY

The recovery of PSG-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	83-118%
Serum (n=4)	91	81-104%
EDTA plasma (n=4)	92	84-108%
Heparin plasma (n=4)	90	83-99%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of PSG-1 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=8)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	106	97	103	102
	Range (%)	103-109	103-119	100-106	101-104
1:4	Average % of Expected	98	107	99	100
	Range (%)	97-100	97-116	96-101	98-102
1:8	Average % of Expected	90	100	95	96
	Range (%)	87-93	90-107	91-96	93-99
1:16	Average % of Expected	85	89	92	90
	Range (%)	82-88	84-96	90-93	88-93

SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of PSG-1 ranged from 0.08-0.48 ng/mL. The mean MDD was 0.23 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human PSG-1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Thirty-five samples from apparently healthy volunteers were evaluated for the presence of PSG-1 in this assay and no detectable levels were observed. No medical histories were available for the donors used in this study.

Cell Culture Supernates - SW480 human colorectal adinocarcinoma cells were cultured in DMEM and supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the culture supernate was removed, and assayed for levels of natural PSG-1, and measured 25.7 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human PSG-1.

The factors listed below were prepared at 2000 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 2000 ng/mL in a mid-range recombinant human PSG-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CEACAM-1
CEACAM-3
CEACAM-4
CEACAM-5
CEACAM-6
CEACAM-7
CEACAM-8
Pappalysin-1/PAPP-A
Pappalysin-2/PAPP-A2
TGF-β1
VEGF-C

Natural proteins:

human TGF-β1

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