Human P-Selectin/CD62P Immunoassay

Catalog Number BBE6
SBBE6
PBBE6

For the quantitative determination of human P-Selectin (P-Selectin) 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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INTRODUCTION

P-Selectin, also known as GMP-140, LECAM-3, PADGEM, and CD62P, is a cell surface glycoprotein that plays a critical role in the migration of lymphocytes into tissues (1-5). It is found constitutively in a pre-formed state in the Weibel-Palade bodies of endothelial cells and in the alpha granules of platelets (4). This stored P-Selectin is mobilized to the cell surface within minutes in response to a variety of inflammatory or thrombogenic agents (4). The mobilized P-Selectin is apparently present on the cell surface for only a few minutes after which it is recycled to intracellular compartments (4). Additional evidence indicates that transcription of P-Selectin mRNA can be activated in the endothelium by treatment with inflammatory mediators (6). P-Selectin consists of an NH2-terminal lectin type C domain, an EGF-like domain, nine complement control domains, a transmembrane domain, and a short cytoplasmic domain (1, 2). Mouse P-Selectin shows a similar organization of functional domains and an overall sequence identity of approximately 73% (5, 6); however, it contains only eight complement control domains, suggesting that the absolute number of these domains is not crucial for function (6). The molecular weight predicted from the cDNA for P-Selectin is approximately 86,000 (1, 2). The observed molecular weight on reducing SDS-PAGE, however, is approximately 140,000 (2).

Evidence indicates that P-Selectin is involved in the adhesion of myeloid cells, B cells and a subset of T cells to activated endothelium (4). P-Selectin is also involved in the adhesion of platelets to monocytes and neutrophils, playing a central role in neutrophil accumulation within thrombi (4). The adhesion of leukocytes and neutrophils to the endothelium is initiated by weak interactions that produce a characteristic "rolling" motion of the leukocytes and neutrophils on the endothelial surface (3-5, 7). P-Selectin, acting in cooperation with L-Selectin, is implicated in the mediation of these initial interactions (3-5, 7). Stronger interactions, probably involving E-Selectin, follow the initial interactions, leading eventually to extravasation through the blood vessel walls into lymphoid tissues and to sites of inflammation (4, 8). The tetrasaccharide sialyl Lewis^x (sLe^x) has been identified as a ligand for both P- and E-Selectin, but P-, E- and L-Selectin can all bind sLe^x and sLe^a under appropriate conditions (4, 5). P-Selectin also reportedly binds selectively to a 160 kDa glycoprotein present on mouse myeloid cells, and to a glycoprotein on human myeloid cells, blood neutrophils, monocytes, and lymphocytes termed P-Selectin glycoprotein ligand-1 (PSGL-1), a ligand that also can bind E-Selectin (4, 8, 9). P-Selectin-mediated rolling of leukocytes can be completely inhibited by a monoclonal antibody specific for PSGL-1, suggesting that even though P-Selectin can bind to a variety of glycoproteins under *in vitro* conditions, it is likely that physiologically important binding is more limited (9).

P-Selectin is found in the plasma of normal individuals at ng/mL concentrations (10). Circulating P-Selectin appears to be slightly smaller than native P-Selectin. An alternatively spliced mRNA encoding a form of human P-Selectin lacking the transmembrane anchoring domain has been reported for both megakaryocytes and endothelial cells (5, 10), and evidence suggests that the majority of circulating soluble P-Selectin (sP-Selectin)arises in this manner (10, 11). A number of studies have reported that levels of sP-Selectin in biological fluids may be elevated in subjects with a variety of pathological conditions (10, 12-16).

This Human P-Selectin/CD62P Immunoassay is a 1.25 hour solid phase ELISA that measures P-Selectin in cell culture supernates, serum, and plasma. It contains recombinant human P-Selectin and antibodies raised against the recombinant factor. It has been shown to accurately quantitate the recombinant P-Selectin. Results obtained using natural human P-Selectin showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural human P-Selectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for human P-Selectin has been pre-coated onto a microplate. Standards, samples and Control are pipetted into the wells together with a polyclonal antibody specific for human P-Selectin which has been conjugated to horseradish peroxidase. Following a wash to remove any unbound conjugated antibody, a substrate is added and color is developed which is proportional to analyte concentration. 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, further dilute the samples with Sample Diluent and repeat the assay.
- Any variation in 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 other factors present in biological samples. Until all factors have been tested in this assay, 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 containers 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.
- Additions at each step of the protocol should be uninterrupted.
- Sodium azide will inactivate the P-Selectin Conjugate.
- Avoid contact of Substrate with oxidizing agents or metal.
- Substrate should remain colorless until added to the plate. Substrate incubated in the positive wells 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # BBE6	CATALOG # SBBE6	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
P-Selectin Microplate	890272	1 plate	6 plates	96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody to human P-Selectin.	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
P-Selectin Standards	890274-890279	1 vial of each level	6 vials of each level	Lyophilized recombinant human P-Selectin with blue dye and preservatives. *The concentrations of P-Selectin after reconstitution are shown on the vial labels.*	May be stored for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
P-Selectin Control	890280	1 vial	6 vials	Lyophilized human serum containing P-Selectin (*see Precautions*). The concentration of the Control should fall within the range specified on the vial label if the assay is valid.	
P-Selectin Conjugate Concentrate	890273	1 vial	6 vials	0.3 mL/vial of a polyclonal antibody to human P-Selectin conjugated to horseradish peroxidase in buffer with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Sample Diluent	895173	2 vials	12 vials	20 mL/bottle of a buffered protein base with blue dye and preservatives.	
Conjugate Diluent	895172	1 vial	6 vials	11 mL/vial of diluent for the HRP-Conjugate concentrate, with red dye and preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Substrate	895002	1 vial	6 vials	11 mL/vial of stabilized substrate solution (tetramethylbenzidine).	
Stop Solution	895004	1 vial	6 vials	11 mL/vial of 1N hydrochloric acid.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

BBE6 contains sufficient materials to run an ELISA on one 96 well plate.
SBBE6 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PBBE6). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm or 650 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.

PRECAUTIONS

The P-Selectin Control contains human serum. This serum was tested at the donor level using FDA licensed methods and was found to be non-reactive for anti-HIV1/2, anti-HCV, HIV-1 antigen, and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, this reagent should be handled as if capable of transmitting infection.

Avoid microbial contamination when removing aliquots from reagent vials.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at 2-8 °C for up to 24 hours or at ≤ -20 °C for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Serum - 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 2-8 °C for up to 1 week or at ≤ -20 °C for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at 2-8 °C for up to 1 week or at ≤ -20 °C for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Notes: *Since P-Selectin is released from platelets (4), values in serum have been found to be significantly different compared to paired plasma samples (See Sample Values section).*

Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

All samples and the P-Selectin Control must be diluted at least 20-fold into Sample Diluent. A suggested 20-fold dilution is 15 µL of sample + 285 µL of Sample Diluent.

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. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Standards - Reconstitute Standards immediately before use with 1.0 mL of distilled or deionized water. Mix by gentle inversion and swirling. Allow vials to sit at room temperature for at least 10 minutes and mix by gentle inversion and swirling until all the contents are completely dissolved. **Vigorous agitation and foaming should be avoided.** The Standards are now ready for use in the assay and require no further dilution. The concentrations of the Standards are stated on the vials.

P-Selectin Control - Reconstitute the P-Selectin Control immediately before use with 500 µL of distilled or deionized water. Allow the Control to sit at room temperature for at least 10 minutes. Mix by gentle inversion and swirling. The P-Selectin Control must be diluted 20-fold in Sample Diluent prior to assay. A suggested 20-fold dilution is 15 µL of Control + 285 µL of Sample Diluent.

P-Selectin Conjugate - Tap the vial of Conjugate Concentrate to dislodge any liquid from the cap. Ensure contents are mixed with a suitable pipette. Transfer 250 µL of the Conjugate Concentrate into the bottle of Conjugate Diluent. Mix by gentle inversion and swirling. **Vigorous agitation and foaming should be avoided.** The P-Selectin Conjugate is now ready for use in the assay and requires no further dilution.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, Standards, and the P-Selectin Control be assayed in duplicate.

1. Prepare all reagents, standards, samples, and Control 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 Standard, Control*, or sample* to each well.
4. Add 100 µL of diluted P-Selectin Conjugate to each well with sufficient force to ensure mixing.
5. Cover the plate with a plate sealer and incubate at room temperature for 1 hour.
6. Aspirate or decant each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid after each wash 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.
7. Add 100 µL of Substrate to each well. Cover the plate with a new plate sealer and incubate at room temperature for 15 minutes.
8. Add 100 µL of Stop Solution to each well. The Stop Solution should be added to the wells in the same order as the Substrate.
9. 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 620 or 650 nm. If wavelength correction is not available, subtract readings at 650 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.

*Samples and the P-Selectin Control require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each Standard, Control, and sample.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph.

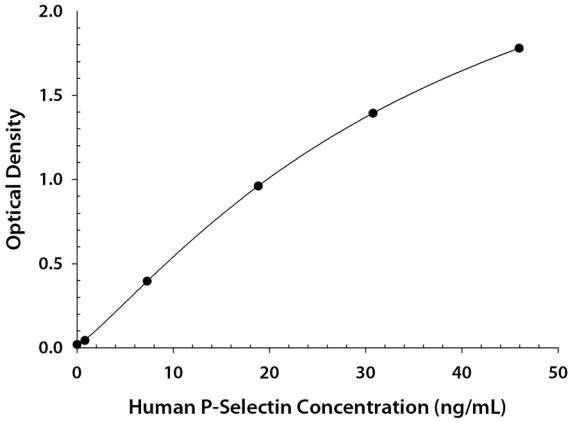
Determine the concentration of each unknown sample by calculating the concentration of P-Selectin corresponding to the mean absorbance from the standard curve.

For samples and the P-Selectin Control, the concentration determined from the standard curve must be multiplied by the dilution factor.

It is recommended that the user run the P-Selectin Control in each assay. If the values obtained are not within the expected range, as stated on the P-Selectin Control vial label, the assay results may be invalid.

TYPICAL DATA

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



Standard	(ng/mL)	O.D.	Average
0	0.00	0.018 0.019	0.018
1	0.82	0.042 0.044	0.043
2	7.28	0.390 0.402	0.396
3	18.83	0.952 0.968	0.960
4	30.76	1.366 1.421	1.394
5	45.94	1.762 1.798	1.780

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested in replicates of ten to assess intra-assay precision.

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

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	10	10	10	18	18	18
Mean (ng/mL)	84	430	668	94	451	730
Standard deviation	4.28	21.1	37.4	9.31	39.7	57.7
CV (%)	5.1	4.9	5.6	9.9	8.8	7.9

RECOVERY

The recovery of P-Selectin spiked to three different levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Serum (n=7)	101	84-119%
EDTA plasma (n=7)	94	78-107%
Heparin plasma (n=7)	100	77-114%
Citrate plasma (n=7)	98	82-111%
Cell culture media (n=5)	105	98-115%

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of P-Selectin were diluted in Sample Diluent and assayed in three batches of kits. Samples were diluted prior to assay as directed in the Sample Preparation section.

		Cell culture supernates (n=5)	Serum (n=7)	EDTA plasma (n=7)	Heparin plasma (n=7)	Citrate plasma (n=7)
1:2	Average % of Expected	97	100	103	101	101
	Range (%)	91-102	93-108	100-110	93-113	92-110
1:4	Average % of Expected	94	100	102	100	99
	Range (%)	87-102	89-111	90-116	90-118	90-114
1:8	Average % of Expected	93	97	104	97	95
	Range (%)	86-99	86-113	89-120	89-119	79-110

HIGH DOSE HOOK

Levels of P-Selectin up to 50,000 ng/mL gave optical density (O.D.) readings greater than the top standard.

SENSITIVITY

The minumum detectable dose (MDD) of human P-Selectin is typically less than 0.5 ng/mL.

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

CALIBRATION

This assay is standardized against a purified soluble form of recombinant human P-Selectin.

SAMPLE VALUES

Serum/Plasma - Matched serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human sP-Selectin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	± 1SD Range (ng/mL)
Serum (n=23)	82	51-113
EDTA Plasma (n=23)	33	22-44
Citrate Plasma (n=23)	29	18-40
Heparin Plasma (n=23)	39	25-53

Note: *The citrate Vacutainers™ which were used contained 0.5 mL of liquid anticoagulant for a 4.5 mL fill. Corrected for the dilution effect of the liquid anticoagulant, the range in citrate plasma was observed to be 20-44 ng/mL.*

SPECIFICITY

This assay recognizes recombinant and natural human P-Selectin. No cross-reactivity or interference was found with recombinant human L-Selectin or recombinant human E-Selectin.

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