# Quantikine® ELISA

## Human P-Selectin/CD62P Immunoassay

Catalog Number DPSE00
SPSE00

For the quantitative determination of human 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).

The Quantikine® Human P-Selectin/CD62P Immunoassay is a 1.25 hour solid-phase ELISA designed to measure human P-Selectin in cell culture supernates, serum, and plasma. It contains recombinant human P-Selectin and antibodies raised against the recombinant factor. Results obtained using natural human P-Selectin 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 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 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 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 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 # DPSE00 | CATALOG # SPSE00 | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|---|---|
| **Human P-Selectin Microplate** | 890272 | 1 plate | 6 plates | 96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for 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.* |
| **Human P-Selectin Standard** | 898963 | 2 vials | 12 vials | Recombinant human P-Selectin in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Use a fresh standard for each assay. Discard after use. |
| **Human P-Selectin Control** | 890280 | 1 vial | 6 vials | Lyophilized human serum containing P-Selectin (*see Precautions*). The assay value of the control should be within the range specified on the label. | May be stored for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles. |
| **Human P-Selectin Conjugate Concentrate** | 890273 | 1 vial | 6 vials | 0.3 mL/vial of a polyclonal antibody specific for human P-Selectin conjugated to horseradish peroxidase in buffer with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **P-Selectin Sample Diluent** | 895173 | 2 vials | 12 vials | 20 mL/bottle of a buffered protein base with blue dye and preservatives. | |
| **P-Selectin 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.* | |
| **Color Reagent A** | 895000 | 1 vial | 6 vials | 12 mL of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 1 vial | 6 vials | 12 mL of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 1 vial | 6 vials | 6 mL of 2 N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 strips | 24 strips | Adhesive strips. | |

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

DPSE00 contains sufficient materials to run an ELISA on one 96 well plate.
SPSE00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

## 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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.

## 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.

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.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

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

## 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 at room temperature 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 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 ≤ -20 °C. 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 Human P-Selectin Control must be diluted at least 20-fold into P-Selectin Sample Diluent. A suggested 20-fold dilution is 15 µL of sample + 285 µL of P-Selectin 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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

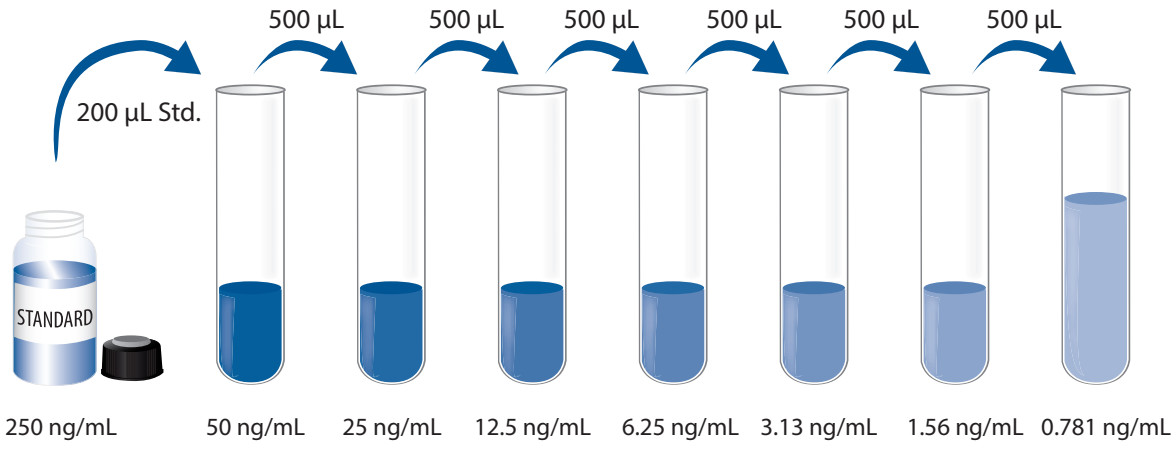
**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

**Human P-Selectin Control** - Reconstitute the P-Selectin Control 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.

**Human P-Selectin Conjugate** - Add 250 µL of the Human P-Selectin Conjugate Concentrate directly to the P-Selectin Conjugate Diluent. Mix by gentle inversion. **Vigorous agitation and foaming should be avoided.**

**Human P-Selectin Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human P-Selectin Standard with deionized or distilled water. This reconstitution produces a stock solution of 250 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Pipette 800 µL of P-Selectin Sample Diluent into the 50 ng/mL tube. Pipette 500 µL of the into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. The P-Selectin Sample Diluent 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, control, and sample be assayed in duplicate.**

1. Prepare all reagents, standards, control, 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 standard, control*, or sample* to each well.
4. Add 100 µL of the diluted Human P-Selectin Conjugate to each well with sufficient force to ensure mixing. Cover with the adhesive strip provided. Incubate for **1 hour** at room temperature.
5. 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 (400 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.
6. Add 200 µL of Substrate Solution to each well. Incubate for 15 minutes at room temperature. **Protect from light.**
7. 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.
8. 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.

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

## CALCULATION OF RESULTS

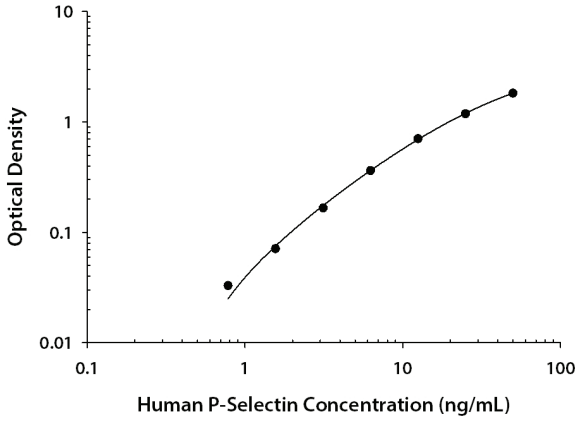
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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. The data may be linearized by plotting the log of the human P-Selectin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since the samples and Human P-Selectin Control 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 | 0.003<br>0.006 | 0.005 | — |
| 0.781 | 0.038<br>0.038 | 0.038 | 0.033 |
| 1.56 | 0.071<br>0.080 | 0.076 | 0.071 |
| 3.13 | 0.166<br>0.175 | 0.171 | 0.166 |
| 6.25 | 0.362<br>0.373 | 0.368 | 0.363 |
| 12.5 | 0.683<br>0.737 | 0.710 | 0.705 |
| 25 | 1.174<br>1.211 | 1.193 | 1.188 |
| 50 | 1.792<br>1.866 | 1.829 | 1.824 |

## 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. Assays were performed by at least three technicians.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **1** | **2** | **3** |
| **n** | 20 | 20 | 20 | 20 | 20 | 20 |
| **Mean (ng/mL)** | 6.54 | 19.3 | 25.6 | 6.51 | 19.9 | 30.5 |
| **Standard deviation** | 0.442 | 1.47 | 2.29 | 0.484 | 1.57 | 3.64 |
| **CV (%)** | 6.8 | 7.6 | 8.9 | 7.4 | 7.9 | 11.9 |

## RECOVERY

The recovery of Human 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 |
|---|---|---|
| Cell culture media (n=4) | 97 | 88-107% |
| Serum (n=4) | 93 | 77-104% |
| EDTA plasma (n=4) | 92 | 77-103% |
| Heparin plasma (n=4) | 95 | 78-112% |
| Citrate plasma (n=4) | 92 | 81-111% |

## HIGH DOSE HOOK

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

## SENSITIVITY

Eighteen assays were evaluated and the minimum detectable dose (MDD) of human P-Selectin ranged from 0.023-0.121 ng/mL. The mean MDD was 0.052 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.

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human P-Selectin were diluted with sample diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

| | | Cell culture media (n=4) | Serum (n=4) | EDTA plasma (n=4) | Heparin plasma (n=4) | Citrate plasma (n=4) |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 99 | 108 | 107 | 105 | 103 |
| | Range (%) | 97-102 | 106-109 | 105-108 | 96-115 | 100-105 |
| 1:4 | Average % of Expected | 99 | 105 | 105 | 104 | 102 |
| | Range (%) | 92-104 | 98-112 | 97-112 | 98-109 | 100-107 |
| 1:8 | Average % of Expected | 96 | 107 | 100 | 102 | 99 |
| | Range (%) | 86-101 | 94-117 | 91-114 | 92-108 | 96-102 |
| 1:16 | Average % of Expected | 98 | 104 | 99 | 99 | 100 |
| | Range (%) | 92-102 | 90-115 | 91-112 | 86-110 | 92-108 |

## CALIBRATION

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

## SAMPLE VALUES

**Serum/Plasma** - Samples from apparently healthy volunteers were evaluated for the presence of human P-Selectin in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean (ng/mL) | Range (ng/mL) | Standard Deviation (ng/mL) |
|---|---|---|---|
| Serum (n=33) | 82.6 | 43.5-119 | 20.0 |
| EDTA Plasma (n=33) | 35.3 | 18.3-57.4 | 8.78 |
| Heparin Plasma (n=33) | 33.7 | 18.6-50.6 | 7.40 |

| Sample Type | Mean of Detectable (ng/mL) | % Detectable | Range (ng/mL) |
|---|---|---|---|
| Citrate plasma (n=33) | 25.8 | 97 | ND-37.9 |

ND=Non-detectable

## SPECIFICITY

This assay recognizes recombinant and natural human P-Selectin.

No cross-reactivity or interference was found with recombinant human E-Selectin, recombinant human L-Selectin, or recombinant mouse P-Selectin.

## REFERENCES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