# Human P-selectin Sandwich ELISA Kit Datasheet

For the quantitative detection of human P-selectin concentrations in serum, plasma and cell culture supernatants.

## General Information

| | |
|---|---|
| **Catalogue Number** | KE00064 |
| **Product Name** | Human P-selectin Sandwich ELISA Kit |
| **Species cross-reactivity** | Human |
| **Range (calibration Range)** | 62.5-4000 pg/mL |
| **Tested applications** | Quantification ELISA |

## Database Links

| | |
|---|---|
| **Entrez Gene** | 6403 |
| **SwissProt** | P16109 |

## Kit Components & Storage

| | | |
|---|---|---|
| **Microplate** - antibody coated 96-well microplate (8 well × 12 strips) | 1 plate | **Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.** |
| **Protein standard** - 8000 pg/bottle; lyophilized* | 2 bottles | |
| **Detection antibody, biotinylated (100X)** - 120 μL/vial | 1 vial | |
| **Streptavidin-horseradish peroxidase (HRP) (100X)** - 120 μL/vial | 1 vial | |
| **Sample Diluent PT 4** - 30 mL/bottle. For serum and plasma samples | 2 bottles | |
| **Sample Diluent PT 4-ef** - 30 mL/bottle. For cell culture supernatants | 1 bottle | |
| **Detection Diluent** - 30 mL/bottle | 1 bottle | |
| **Wash Buffer Concentrate (20X)** - 30 mL/bottle | 1 bottle | |
| **Tetramethylbenzidine Substrate (TMB)** - 12 mL/bottle | 1 bottle | |
| **Stop Solution** - 12 mL/bottle | 1 bottle | |
| **Plate Cover Seals** | 3 pieces | |



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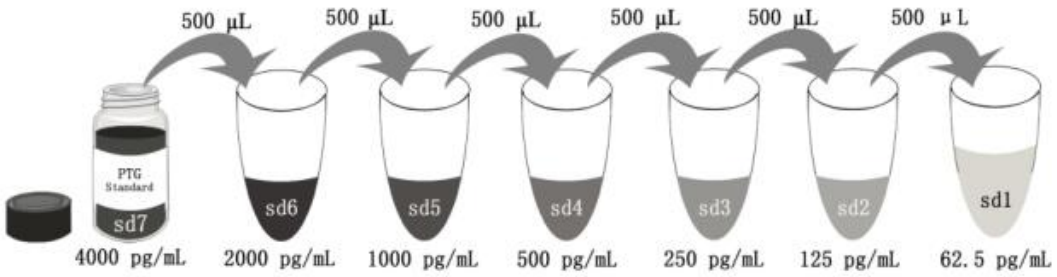
**NB: Do not use the kit after the expiration date.**
Sample Diluent PT 4 is for protein standard, serum and plasma samples.
Sample Diluent PT 4-ef is for protein standard and cell culture supernatants.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 4 or PT 4-ef in protein standard. This reconstitution gives a stock solution of 4000 pg/mL.




| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
|---|---|---|---|---|---|---|---|
| # µL of Sample Diluent PT 4 or PT 4-ef | **2000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

## Product Description

KE00064 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The SELP ELISA kit is to be used to detect and quantify protein levels of endogenous SELP. The assay recognizes human SELP. An antibody specific for SELP has been pre-coated onto the microwells. The SELP protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for SELP is added to detect the captured SELP protein. For signal development, Streptavidin-HRP is added is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## Background

SELP, also named as P-selectin, GMP-140 or CD62P, is a transmembrane glycoprotein that mediates the interaction of activated endothelial cells or platelets with leukocytes. It is an adhesion molecule involved in the pathogenesis of inflammation, thrombosis, and oncogenesis. P-selectin is stored in the alpha-granules of platelets and Weibel-Palade bodies of endothelial cells. Upon cell activation by agonists, P-selectin is transported rapidly to the cell surface. Soluble P-selectin is produced by platelets and endothelial cells and circulates in normal plasma.

## Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. The serum is better to be diluted 1:300 before assay, the plasma is better to be diluted 1:100 and 1:2 dilution is recommended for cell culture



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supernatants.

## Safety Notes

This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

| Step | Reagent | Volume | Incubation | Wash | Notes |
|---|---|---|---|---|---|
| 1 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Antibody Solution | 100 µL | 60 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent HRP Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |



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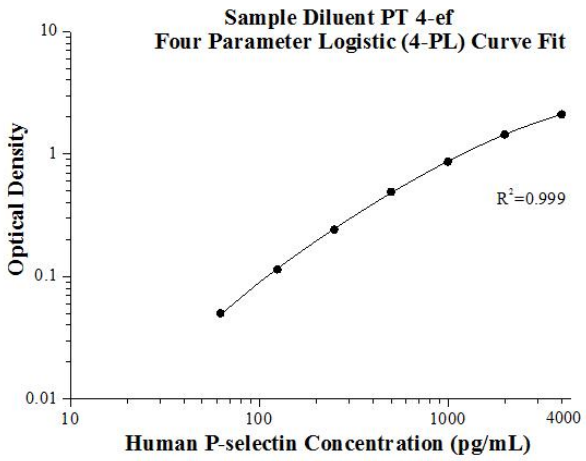
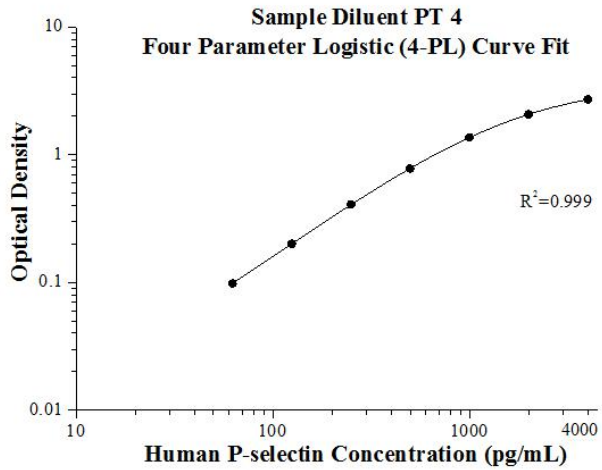
## Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.051<br>0.052 | 0.052 | - |
| 62.5 | 0.103<br>0.101 | 0.102 | 0.050 |
| 125 | 0.169<br>0.163 | 0.166 | 0.114 |
| 250 | 0.295<br>0.291 | 0.293 | 0.241 |
| 500 | 0.542<br>0.54 | 0.541 | 0.489 |
| 1000 | 0.914<br>0.913 | 0.914 | 0.862 |
| 2000 | 1.5<br>1.484 | 1.492 | 1.440 |
| 4000 | 2.158<br>2.142 | 2.150 | 2.098 |




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.082<br>0.081 | 0.082 | - |
| 62.5 | 0.178<br>0.181 | 0.180 | 0.098 |
| 125 | 0.283<br>0.281 | 0.282 | 0.200 |
| 250 | 0.491<br>0.486 | 0.489 | 0.407 |
| 500 | 0.853<br>0.865 | 0.859 | 0.777 |
| 1000 | 1.445<br>1.445 | 1.445 | 1.363 |
| 2000 | 2.144<br>2.148 | 2.146 | 2.064 |
| 4000 | 2.786<br>2.775 | 2.781 | 2.699 |



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## Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 20 | 1,878.5 | 46.1 | 2.5 |
| 2 | 20 | 483.9 | 14.9 | 3.1 |
| 3 | 20 | 118.6 | 6.2 | 5.2 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 20 | 1,983.3 | 75.9 | 3.8 |
| 2 | 20 | 479.6 | 18.6 | 3.9 |
| 3 | 20 | 104.6 | 6.4 | 6.1 |

## Recovery

The recovery of P-selectin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Human serum | 1:600 | 95 | 78-107 |
| | 1:1,200 | 99 | 87-102 |
| Human plasma | 1:100 | 97 | 85-104 |
| | 1:200 | 104 | 101-109 |
| Cell culture supernatants | 1:2 | 95 | 84-113 |
| | 1:4 | 94 | 86-108 |

## Sample Values

Serum and plasma samples from healthy volunteers were evaluated for P-selectin in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean of Detectable (ng/mL) | Range (ng/mL) |
|---|---|---|
| Human serum (n=24) | 179 | 22 - 552 |
| Human plasma (n=24) | 211 | 32 - 339 |

## Sensitivity

The minimum detectable dose of human P-selectin is 4.7 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.



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## Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of P-selectin in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:50. The serum samples were initially diluted 1:150)

| | | Human plasma (Sample Diuent PT 4) | Serum (Sample Diuent PT 4) | Cell culture supernatants (Sample Diuent PT 4-ef) |
|---|---|---|---|---|
| 1:2 | Average% of Expected | 100 | 100 | 106 |
| | Range (%) | - | - | 98-114 |
| 1:4 | Average% of Expected | 100 | 100 | 108 |
| | Range (%) | 91-107 | 92-106 | 100-117 |
| 1:8 | Average% of Expected | 103 | 101 | 111 |
| | Range (%) | 97-109 | 95-113 | 103-118 |
| 1:16 | Average% of Expected | 106 | 103 | 107 |
| | Range (%) | 99-117 | 96-115 | 98-117 |

## References

1. Koedam JA, et al. P-selectin, a granule membrane protein of platelets and endothelial cells, follows the regulated secretory pathway in AtT-20 cells. J Cell Biol. 116(3):617-25 (1992).
2. Dunlop LC, et al. Characterization of GMP-140 (P-selectin) as a circulating plasma protein. J Exp Med. 175(4):1147-50 (1992).
3. Davì G, et al. Increased levels of soluble P-selectin in hypercholesterolemic patients. Circulation. 97(10):953-7 (1998).
4. Pasquali A, et al. Detection of a large deletion in the P-selectin (SELP) gene. Mol Cell Probes. 24(3):161-5 (2010).
5. Panicker SR, et al. Circulating soluble P-selectin must dimerize to promote inflammation and coagulation in mice. Blood. 130(2):181-191 (2017).



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