

## Instructions for Use



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# sE-Selectin ELISA

Enzyme immunoassay for the quantitative or qualitative determination of soluble human E-selectin in cell culture supernatants, serum and plasma (citrate and heparin).

**REF** 30149799

 96

  2°C  8°C

**For research use only.**  
**Not for use in diagnostic procedures.**



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**Always there for you**



## 1. INTENDED USE

The sE-Selectin ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sE-selectin. **For research use only. Not for diagnostic or therapeutic procedures.**

## 2. SUMMARY

Endothelial Leukocyte Adhesion Molecule-1 (ELAM-1, E-selectin) belongs to the selectin family of adhesion molecules. Together with LECAM-1 (L-selectin) and GMP-140 (P-selectin), E-selectin mediates the initial interactions of leukocytes and platelets with endothelial cells.

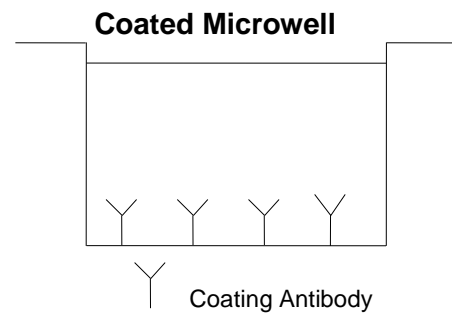
Molecular structure: The extracellular part of all selectins consists of an aminoterminal c-type lectin domain that specifically binds to carbohydrate ligands. This is followed by an EGF-like domain, and, in the case of E-selectin, by 6 short consensus repeats. The transmembrane portion of the molecule is followed by a short cytoplasmic tail.

Selectins guide nonactivated polymorphonuclear cells to the areas of inflammation in creating first, loose contacts with the endothelial layer. The potential binding partner of E-selectin contains sialyl LewisX oligosaccharide. Other suitable ligands for the lectin domain of E-selectin are sialylated, fucosylated lactosaminoglycans. Together with GMP-140, E-selectin is expressed on cytokine-activated endothelial cells, and contributes to the adhesion of still resting leukocytes to the endothelium. This initial binding event is an essential prerequisite for the activation of the immune cells via different inflammatory mediators. In contrast to GMP-140, E-selectin is maximally expressed 2–4 hours after cell activation. Within the next 24–48 hours E-selectin is again eliminated from the cytoplasmic membrane by shedding into the circulation.

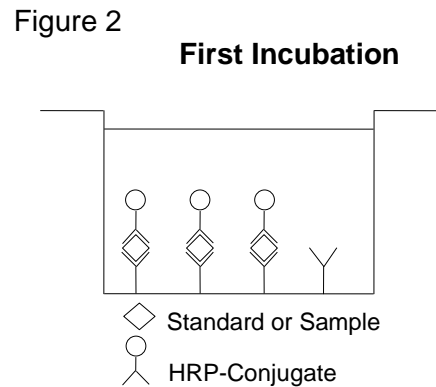
The circulating form or soluble (sE-selectin) of this selectin exerts chemotactical signals on neutrophils and additionally activates the 2- integrins—sE-selectin assists in preparing the migration capacity of these cells.

### 3. PRINCIPLES OF THE TEST

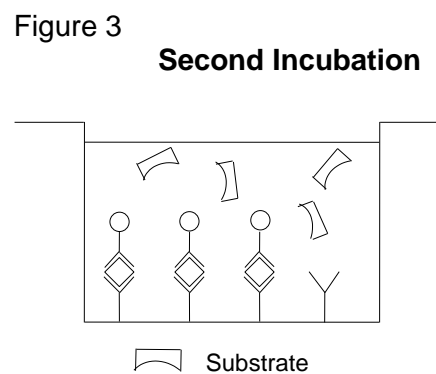
An anti-human sE-selectin coating antibody is adsorbed onto microwells. Figure 1



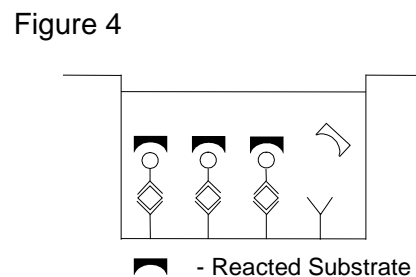
Human sE-selectin present in the sample or standard binds to antibodies adsorbed to the microwells. A HRP-conjugated anti-human E-selectin antibody is added and binds to human sE-selectin captured by the first antibody. Figure 2



Following incubation unbound HRP-conjugated anti-human sE-selectin is removed during a wash step, and substrate solution reactive with HRP is added to the wells. Figure 3



A colored product is formed in proportion to the amount of human sE-selectin present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 6 human sE-selectin standard dilutions and human sE-selectin concentration determined. Figure 4



#### 4. REAGENTS PROVIDED

<b>MTP</b>	1 aluminium pouch with a <b>Microwell Plate</b> (12 strips of 8 wells each) <b>coated</b> with monoclonal antibody to human sE-selectin
<b>ENZCONJ</b> <b>CONC</b>	1 vial (150 µL) <b>HRP-Conjugate</b> anti-human sE-selectin monoclonal antibody
<b>CAL</b> <b>LYO</b>	2 vials human sE-selectin <b>Standard</b> lyophilized, 100 ng/mL upon reconstitution
<b>CONTROL L</b> <b>LYO</b>	1 vial <b>Control Low</b> , lyophilized
<b>CONTROL H</b> <b>LYO</b>	1 vial <b>Control High</b> , lyophilized
<b>SUBS</b>	1 vial (15 mL) <b>Substrate Solution</b> (tetramethyl-benzidine)
<b>WASHBUF</b> <b>CONC</b>	1 bottle (50 mL) <b>Wash Buffer Concentrate</b> 20x (PBS with 1% Tween 20)
<b>ASSAYBUF</b> <b>CONC</b>	1 vial (5 mL) <b>Assay Buffer Concentrate</b> 20x (PBS with 1% Tween 20; 10% BSA)
<b>SAMPLEDIL</b>	1 vial (12 mL) <b>Sample Diluent</b>
<b>STOP</b>	1 vial (15 mL) <b>Stop Solution</b> (1M Phosphoric acid)
	2 <b>Adhesive Films</b>

#### 5. STORAGE INSTRUCTIONS – ELISA KIT

Store kit reagents between 2°C and 8°C except controls. Store lyophilized controls at -20°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C), controls to -20°C, respectively. Expiry of the kit and reagents is stated on labels.

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, this reagent is not contaminated by the first handling.

#### 6. SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma (citrate and heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells 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 samples.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human E-selectin. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (refer to "Sample stability").

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

#### 7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks and 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 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 statement(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 pipete 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 samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates 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 samples and all potentially contaminated materials if as 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

1. Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

### 9.1. Wash Buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2°C to 25°C. The Wash Buffer (1x) is stable for 30 days.
3. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

### 9.2. Assay Buffer (1x)

1. Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
2. Store at 2° to 8°C. The Assay Buffer (1x) is stable for 30 days.
3. Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

### 9.3. HPR-Conjugate

The Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated HPR-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

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

### 9.4. Human sE-selectin Standard

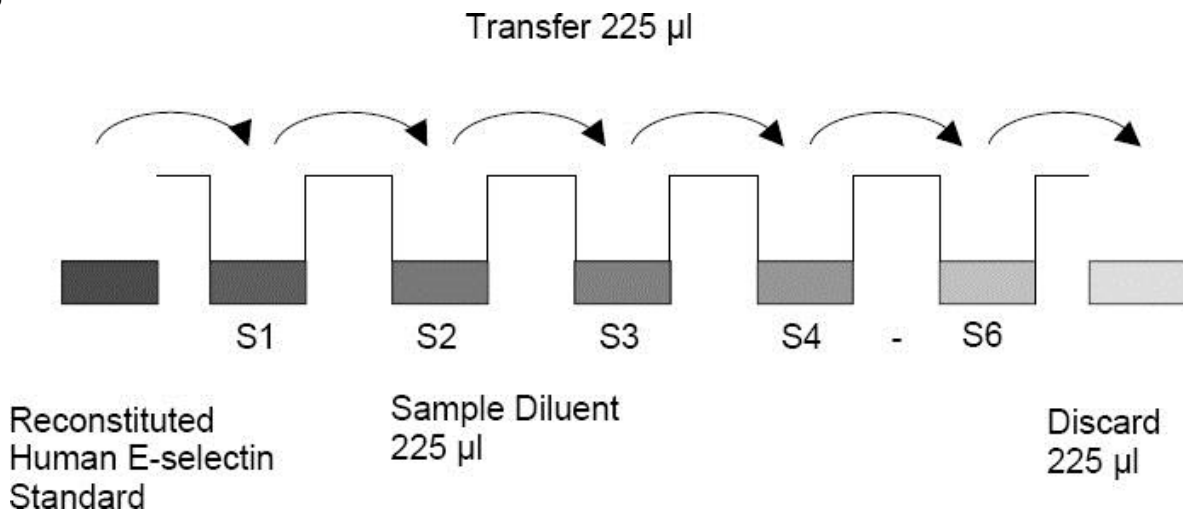
4. Reconstitute human sE-selectin standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 100 ng/mL).
5. Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

The standard has to be used immediately after reconstitution and cannot be stored.

#### 9.4.1. External Standard Dilution

1. Label 6 tubes, one for each standard point.: S1, S2, S3, S4, S5, S6.
2. Then prepare 1:2 serial dilutions for the standard curve as follows:  
Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 100 ng/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 50 ng/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 5).  
Sample Diluent serves as blank.

Figure 5



### 9.5. Controls

Reconstitute lyophilized controls by addition of distilled water (10-30 minutes). Reconstitution volume is stated on the label of the control vial. Swirl or mix gently to ensure complete and homogeneous solubilization. Further treat the controls like your samples in the assay. For control range please refer to certificate of analysis. Store reconstituted controls aliquoted at  $-20^{\circ}\text{C}$ . Avoid repeated freeze and thaw cycles.

## 10. TEST PROTOCOL

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

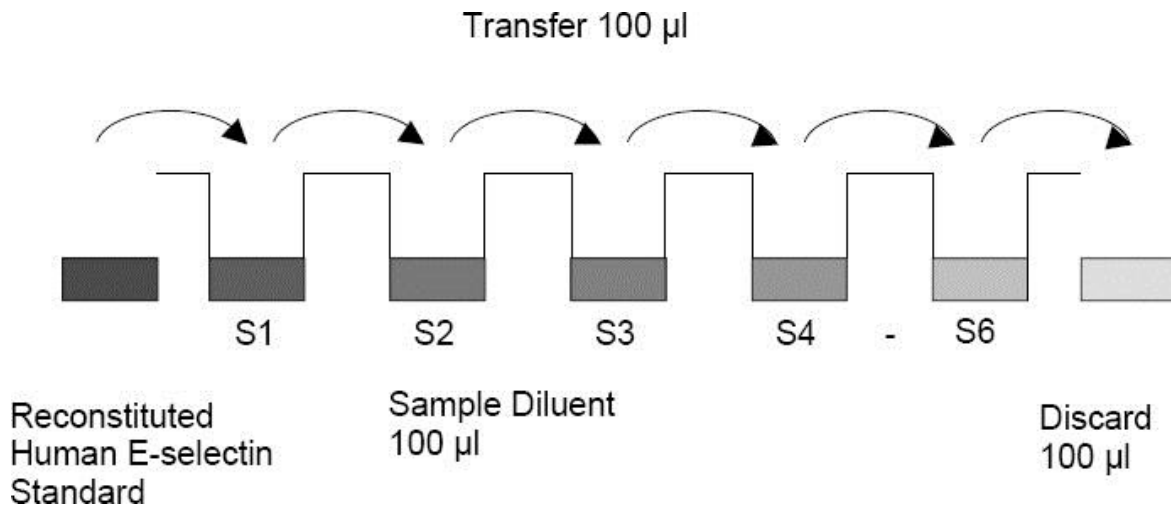
- 1 Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at  $2^{\circ}\text{C} - 8^{\circ}\text{C}$  sealed tightly.
- 2 Wash the microwell strips twice with approximately 400  $\mu\text{L}$  Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.

After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

- 3 Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes - see "External standard dilution"):

Add 100  $\mu\text{L}$  of Sample Diluent in duplicate to all standard wells. Pipet 100  $\mu\text{L}$  of prepared standard (see "sE-selectin standard", concentration = 100.0 ng/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 50.0 ng/mL), and transfer 100  $\mu\text{L}$  to wells B1 and B2, respectively (see Figure 6). Take care not to scratch the inner surface of the microwells. Continue this procedure 4 times, creating two rows of sE-selectin standard dilutions ranging from 50.0 to 1.6 ng/mL. Discard 100  $\mu\text{L}$  of the contents from the last microwells (F1, F2) used.

Figure 6



In case of an external standard dilution (see “External standard dilution”), pipet 100  $\mu$ L of these standard dilutions (S1– S6) in the standard wells according to Table 1.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
<b>A</b>	Standard 1 (50.0 ng/mL)	Standard 1 (50.0 ng/mL)	Sample 2	Sample 2
<b>B</b>	Standard 2 (25.0 ng/mL)	Standard 2 (25.0 ng/mL)	Sample 3	Sample 3
<b>C</b>	Standard 3 (12.5 ng/mL)	Standard 3 (12.5 ng/mL)	Sample 4	Sample 4
<b>D</b>	Standard 4 (6.3 ng/mL)	Standard 4 (6.3 ng/mL)	Sample 5	Sample 5
<b>E</b>	Standard 5 (3.1 ng/mL)	Standard 5 (3.1 ng/mL)	Sample 6	Sample 6
<b>F</b>	Standard 6 (1.6 ng/mL)	Standard 6 (1.6 ng/mL)	Sample 7	Sample 7
<b>G</b>	Blank	Blank	Sample 8	Sample 8
<b>H</b>	Sample 1	Sample 1	Sample 9	Sample 9

- 4 Add 100  $\mu$ L of Sample Diluent in duplicate to the blank wells.
- 5 Add 80  $\mu$ L of Sample Diluent to the sample wells.
- 6 Add 20  $\mu$ L of each sample in duplicate to the sample wells.
- 7 Prepare HRP-Conjugate (see “HRP-Conjugate“ on page 3).
- 8 Add 50  $\mu$ L of HRP-Conjugate to all wells.
- 9 Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 2 hours on a microplate shaker.
- 10 Remove adhesive film and empty wells. Wash microwell strips 5 times according to step 2. Proceed immediately to the next step.
- 11 Pipet 100  $\mu$ L of TMB Substrate Solution to all wells.
- 12 Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm.

The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

- 13 Stop the enzyme reaction by quickly pipetting 100  $\mu$ L of Stop Solution into each well. 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°C to 8°C in the dark.
- 14 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 standards.

## 11. CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 % of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the sE-selectin concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human sE-selectin 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 human sE-selectin concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:5 (20  $\mu$ L sample + 80  $\mu$ L Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 5).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human sE selectin levels. Such samples require further external predilution according to expected human sE selectin values with Sample Diluent in order to precisely quantitate the actual human sE selectin level.
- It is suggested that each testing facility establishes a control sample of known human sE-selectin concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 7.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 7

Representative standard curve for sE-selectin ELISA. Human sE-selectin was diluted in serial 2-fold steps in Sample Diluent.

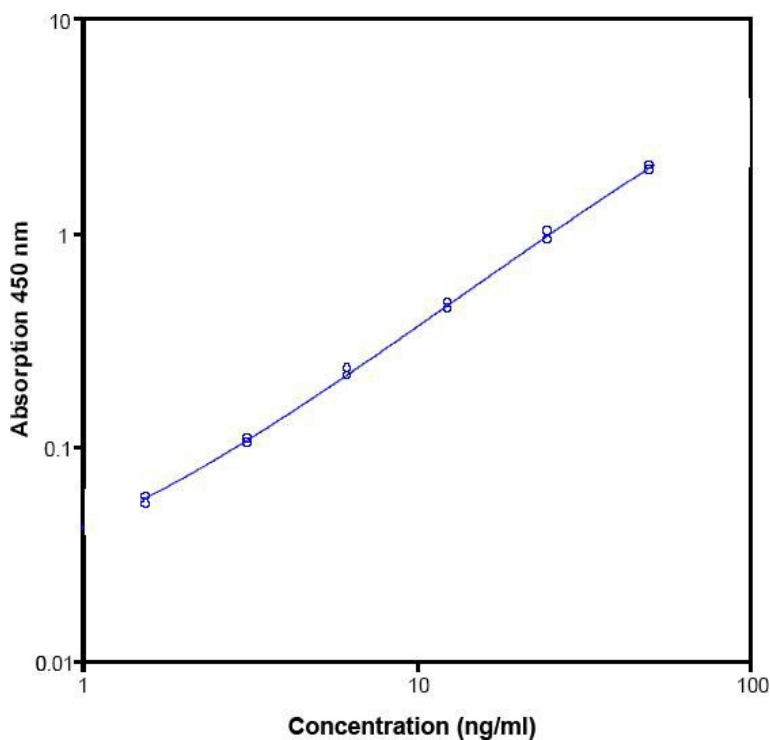


Table 2

Typical data using the sE-selectin ELISA.

Measuring wavelength: 450 nm Reference wavelength: 620 nm.

Standard	Human sE- selectin concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	50.0	2.045 1.936	1.991	3.9
2	25.0	1.018 0.919	0.969	7.3
3	12.5	0.471 0.441	0.456	4.7
4	6.3	0.230 0.213	0.222	5.6
5	3.1	0.109 0.103	0.106	4.3
6	1.6	0.058 0.054	0.056	6.0
Blank	0.0	0.008 0.008	0.008	0.0

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).

Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

## 12. LIMITATIONS

- Because 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. Empty wells completely before dispensing fresh wash solution, 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 radioimmunotherapy has significantly increased the number of samples with human anti-mouse IgG antibodies (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 analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

### 13. PERFORMANCE CHARACTERISTICS

#### 13.1. Sensitivity

The limit of detection of human sE-selectin defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.3 ng/mL (mean of 6 independent assays).

#### 13.2. Reproducibility

##### 13.2.1. Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human sE-selectin. Two standard curves were run on each plate. Data below show the mean human sE-selectin concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 5.4%.

Table 3 The mean human sE-selectin concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human sE-selectin Concentration (pg/mL)	Coefficient of Variation (%)
1	1	32.3	5.1
	2	33.7	4.6
	3	38.3	5.6
2	1	35.8	3.6
	2	35.4	6.1
	3	38.7	5.8
3	1	37.1	3.6
	2	36.2	7.2
	3	39.8	3.0
4	1	30.3	4.9
	2	28.0	2.2
	3	30.0	2.7
5	1	34.4	3.3
	2	40.1	3.4
	3	35.7	4.7
6	1	12.3	8.5
	2	12.1	7.7
	3	12.3	4.7
7	1	23.7	4.7
	2	22.2	8.7
	3	24.5	6.9
8	1	19.6	8.0
	2	17.2	6.1
	3	22.9	7.6

### 13.2.2. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human sE- selectin. Two standard curves were run on each plate. Data below show the mean human sE-selectin concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 6.0%.

Table 4 The mean human sE-selectin concentration and the coefficient of variation of each sample

Sample	Mean Human sE-selectin Concentration (pg/mL)	Coefficient of Variation (%)
1	34.8	9.1
2	36.6	4.9
3	37.7	4.9
4	29.4	4.3
5	36.7	8.1
6	12.3	0.9
7	23.5	5.1
8	19.9	14.4

### 13.3. Spike Recovery

The spike recovery was evaluated by spiking 3 levels of human E- selectin into serum samples. Recoveries were determined in 3 independent experiments with 4 replicates each.

The amount of endogenous human sE-selectin in unspiked serum was subtracted from the spike values. The recovery ranged from 71% to 95% with an overall mean recovery of 86%.

### 13.4. Dilution Parallelism

Four serum samples with different levels of human sE-selectin were analyzed at serial 2 fold dilutions with 4 replicates each. The recovery ranged from 89% to 106% with an overall recovery of 95% (see Table 5).

Table 5

Sample	Dilution	Expected Human E-selectin Concentration (ng/mL)	Observed Human E-selectin Concentration (ng/mL)	Recovery of Expected Human E-selectin Concentration (%)
1	1:5	--	52.8	--
	1:10	26.4	25.3	96
	1:20	12.6	11.6	92
	1:40	5.8	6.1	105
2	1:5	--	36.5	--
	1:10	18.2	18.5	102
	1:20	9.3	8.3	89
	1:40	4.1	3.7	89
3	1:5	--	51.1	--
	1:10	25.5	23.4	92
	1:20	11.7	10.6	91
	1:40	5.3	5.0	94
4	1:5	--	48.9	--
	1:10	24.5	22.6	92
	1:20	11.3	10.3	92
	1:40	5.2	5.5	106

### 13.5. Sample Stability

#### 13.5.1. Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human sE-selectin levels determined. There was no significant loss of human sE-selectin immunoreactivity detected by freezing and thawing.

#### 13.5.2. Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2- 8°C, room temperature and at 37°C, and the human sE-selectin level determined after 24 hours. There was no significant loss of human sE-selectin immunoreactivity detected during storage under above conditions.

### 13.6. Comparison of Serum and Plasma

From several individuals serum as well as citrate and heparin plasma obtained at the same time point were evaluated. Human sE-selectin 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.

### 13.7. Specificity

The assay detects both natural and recombinant human sE-selectin. The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human sE-selectin positive serum. No cross-reactivity was detected, namely not with IL-8, sICAM-1, TNF-R, TNF $\beta$ , CD8, IL-2R, IL-6, L-selectin, and P-selectin.

## 14. REAGENT PREPARATION SUMMARY

### 14.1. Wash Buffer (1x)

Add Wash Buffer Concentrate 20x (50 mL) to 950 mL distilled water.

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

### 14.2. Assay Buffer (1x)

Add Assay Buffer Concentrate 20x (5 mL) to 95 mL distilled water.

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

### 14.3. HPR-Conjugate

Make a 1:100 dilution of HPR-Conjugate in Assay Buffer (1x):

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

### 14.4. Human sE-selectin Standard

Reconstitute lyophilized sE-selectin standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

### 14.5. Controls

Reconstitute lyophilized controls by addition of distilled water (10-30 minutes).










Reconstitution volume is stated on the label of the control vial.

## 15. TEST PROTOCOL SUMMARY

Note: If instructions in this protocol have been followed, samples have been diluted 1:5 (20  $\mu$ L sample + 80  $\mu$ L Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 5).

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100  $\mu$ L Sample Diluent, in duplicate, to all standard wells. Pipet 100  $\mu$ L prepared standard into the first wells and create standard dilutions by transferring 100  $\mu$ L from well to well. Discard 100  $\mu$ L from the last wells.  
Alternatively, external standard dilution in tubes (see "External standard dilution"):  
Pipet 100  $\mu$ L of these standard dilutions in the microwell strips.
4. Add 100  $\mu$ L Sample Diluent, in duplicate, to the blank wells.
5. Add 80  $\mu$ L Sample Diluent to sample wells.
6. Add 20  $\mu$ L sample in duplicate, to designated sample wells.
7. Prepare the HRP-Conjugate.
8. Add 50  $\mu$ L HRP-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18°C to 25°C).
10. Empty and wash microwell strips 5 times with Wash Buffer.
11. Add 100  $\mu$ L of TMB Substrate Solution to all wells.
12. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
13. Add 100  $\mu$ L Stop Solution to all wells.
14. Blank microwell reader and measure color intensity at 450 nm.

# Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED.</p> <p>Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.</p> <p>Voir MATERIEL FOURNI pour les symbôles des composants du kit.</p> <p>Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.</p> <p>Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.</p> <p>Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.</p> <p>Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

**COMPLAINTS:** Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

**WARRANTY:** The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

**LIMITATION OF LIABILITY:** IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.

For further country-specific classifications, please refer to the corresponding safety data sheet.



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