PRODUCT INFORMATION & MANUAL

Human sE-selectin Instant ELISA

BMS205INST

Enzyme-linked immunosorbent assay for quantitative detection of human sE-selectin.
For research use only.
Not for diagnostic or therapeutic procedures.
128 Tests

eBioscience®
An Affymetrix Company

Human sE-selectin Instant ELISA

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1 Intended Use

The human sE-selectin Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sE-selectin. **The human sE-selectin Instant ELISA is 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 which 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 non-activated 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.

Determination of sE-selectin could provide more detailed insights into the pathological modifications during various diseases:

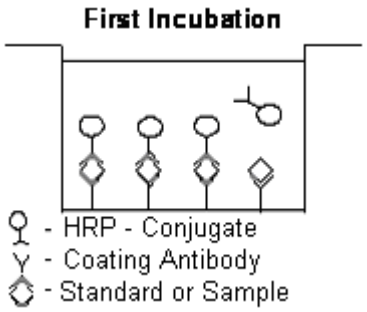
- allergic reactions: the transient influx of neutrophils into the respiratory tract, due to an inflammatory response is predominantly mediated via E-selectin. A functional role for this molecule in the development of acute airway inflammation in vivo has been demonstrated. Additionally, E-selectin may be of particular importance for the start phase of allergic contact dermatitis.
- ocular diseases: the presents of E-selectin on retinal vascular endothelium suggests an important role for this selectin in the pathogenesis of immunologically mediated ocular conditions.
- septic shock: E-selectin seems to be involved in the pathogenesis of "multiple organ failure (MOF)" during septic shock.
- vascular infection and inflammation: The levels of sE-selectin in patients with a recent onset of giant cell arteriitis or polyarteriitis nodosa are significantly higher than in normal controls.
- inflammatory bowel disease: E-selectin is expressed on colonic endothelial surfaces in association with inflammation.
- transplantation: Increased E-selectin expression on endothelial cells is found in graft-versus-host-disease.

For literature update refer to **www.eBioscience.com**

3 Principles of the Test

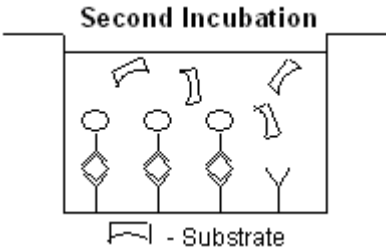
An anti-human sE-selectin coating antibody is adsorbed onto microwells. Human sE-selectin present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated anti-human sE-selectin antibody binds to human sE-selectin captured by the first antibody.

Figure 1



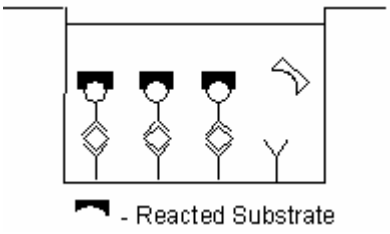
Following incubation unbound enzyme conjugated anti-human sE-selectin is removed during a wash step and substrate solution reactive with HRP is added to the wells.

Figure 2



A coloured product is formed in proportion to the amount of human sE-selectin present in the sample. 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 sample concentration determined.

Figure 3



4 Reagents Provided

1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human sE-selectin, **HRP-Conjugate** (human sE-selectin monoclonal antibody) and Sample Diluent, lyophilized

2 aluminium pouches with a human sE-selectin **Standard curve** (coloured)

1 bottle (25 ml) **Wash Buffer Concentrate** 20x
(phosphate-buffered saline with 1% Tween 20)

1 vial (12 ml) **Sample Diluent**
(Use when an external predilution of the samples is needed)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate and standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.
The 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, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatant, serum and plasma (citrate, 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 specimens. Centrifugation of lipemic samples (16.000 x g for 5 min) before analysis is necessary.

Samples must be stored frozen at -20°C to avoid loss of bioactive human sE-selectin. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).
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
- adjustable multichannel micropipettes (for volumes between 50 µl and 500 µl) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Centrifuge
- 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 linear regression analysis

8 Precautions for Use

- All reagents 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 statements(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 pipette 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 specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- 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 specimens and all potentially contaminated materials 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 and Samples

Buffer concentrate should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in the buffer concentrate, warm it gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer is stable for 30 days.

10 Test Protocol

- **Use plate immediately after removal from -20°C!**
- **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
- **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.**
- **Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.**
- **Allow the washing buffer to sit in the wells for a few seconds before aspiration.**
- **Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.**

a. Determine the number of microwell strips required to test the desired number of samples plus microwell strips for blanks and standards (coloured). Each sample, standard and blank should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).

b. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).

c. Add 130 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (50.00 ng/ml)	Standard 1 (50.00 ng/ml)	Sample 1	Sample 1
B	Standard 2 (25.00 ng/ml)	Standard 2 (25.00 ng/ml)	Sample 2	Sample 2
C	Standard 3 (12.50 ng/ml)	Standard 3 (12.50 ng/ml)	Sample 3	Sample 3
D	Standard 4 (6.25 ng/ml)	Standard 4 (6.25 ng/ml)	Sample 4	Sample 4
E	Standard 5 (3.13 ng/ml)	Standard 5 (3.13 ng/ml)	Sample 5	Sample 5
F	Standard 6 (1.56 ng/ml)	Standard 6 (1.56 ng/ml)	Sample 6	Sample 6
G	Blank	Blank	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 20 µl of each **sample**, in duplicate, to the **designated wells** and mix the contents.

e. Cover with an adhesive film and incubate at room temperature (18ºC to 25ºC) for 3 hours, on a microplate shaker at 400 rpm. (Shaking is absolutely necessary for an optimal test performance.)

f. Remove adhesive film and empty wells. **Wash** the microwell strips 3 times with approximately **400 µl** 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, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

g. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.

h. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

   The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour 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 colour. Alternatively the colour 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.

i. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. 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 - 8°C in the dark.

j. 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 human sE-selectin standards.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human 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.
- ***Samples have been diluted 1:5, thus 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 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µl to the final volume per well. These 100 µl are composed of 80 µl of Sample Diluent plus 20 µl of the sample. This is a 1:5 dilution.
The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.
80 µl Sample Diluent and 50 µl conjugate results in 130 µl reconstitution volume, addition of 20 µl sample (80 µl + 20 µl sample= 1:5 dilution)

Figure 4
Representative standard curve for human sE-selectin Instant ELISA. Human sE-selectin was diluted in serial 2-fold steps in Sample Diluent. Each symbol represents the mean of 3 parallel titrations.
Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

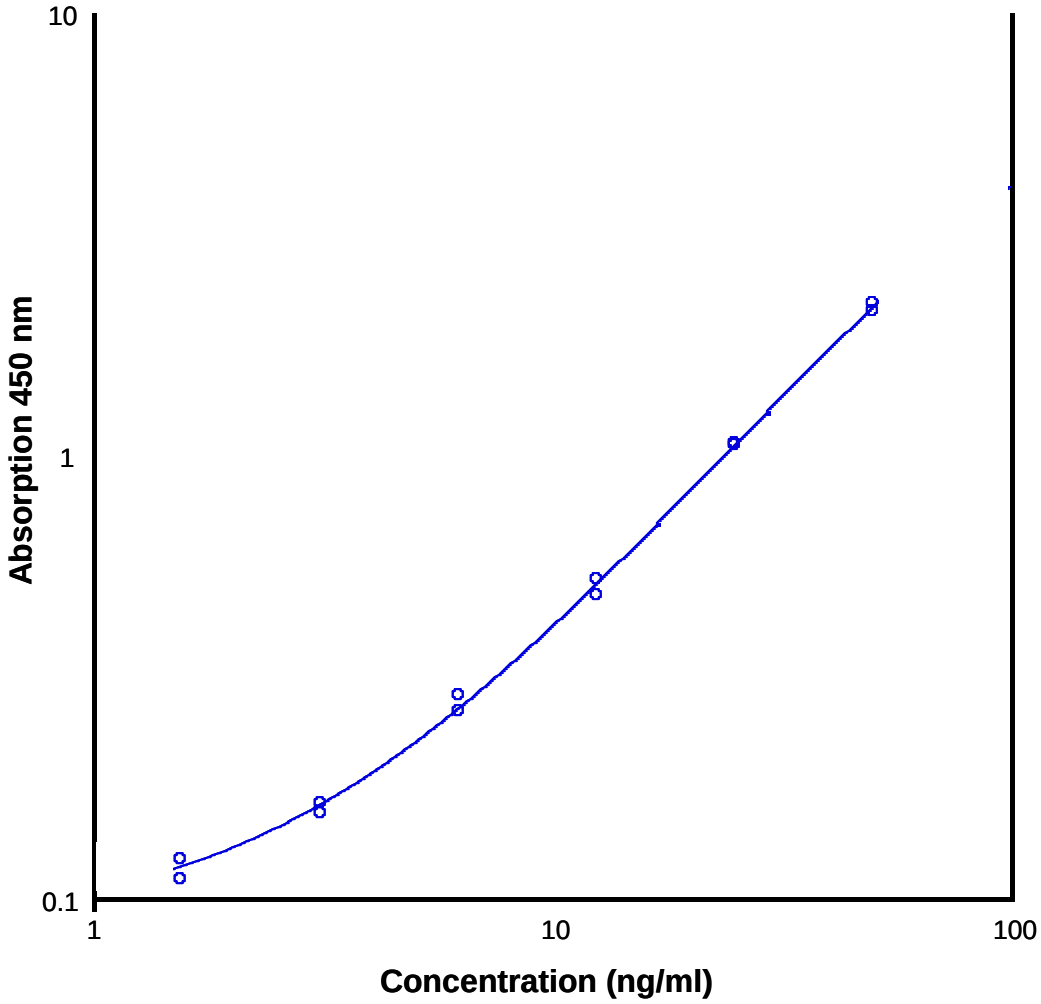


Table 2
Typical data using the human sE-selectin INSTANT ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human sE-selectin Concentration (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	50.00	2.182	2.147	2.3
		2.111		
2	25.00	1.046	1.050	0.5
		1.054		
3	12.50	0.524	0.502	6.2
		0.480		
4	6.30	0.286	0.275	5.7
		0.264		
5	3.10	0.155	0.159	3.6
		0.163		
6	1.60	0.110	0.116	7.3
		0.122		
Blank	0.00	0.045	0.047	0.0
		0.048		

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 colour intensity. Values measured are still valid.

12 Limitations

- Since 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 patients 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 analysed 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 0.33 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 6 serum samples containing different concentrations of human sE-selectin. 2 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 6.9%.

Table 3

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

Sample	Experiment	Human sE-selectin Concentration (ng/ml)	Coefficient of Variation (%)
1	1	124	7.8
	2	153	7.5
	3	142	4.7
2	1	164	8.2
	2	196	7.9
	3	193	3.3
3	1	301	8.0
	2	294	5.5
	3	302	7.1
4	1	256	6.2
	2	274	9.9
	3	248	8.0
5	1	216	6.9
	2	260	10.1
	3	240	5.8
6	1	195	8.8
	2	230	2.4
	3	197	6.3

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 6 serum samples containing different concentrations of human sE-selectin. 2 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 7.5%.

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

Sample	Mean human sE-selectin Concentration (ng/ml)	Coefficient of Variation (%)
1	140	6.7
2	184	6.5
3	299	6.9
4	259	8.0
5	239	7.6
6	207	5.8

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human sE-selectin into serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments.
Average recovery ranged from 85% to 126% with an overall mean recovery of 109%.

13.4 Dilution Parallelism

4 serum samples with different levels of human sE-selectin were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 89% and 122% with an overall recovery of 113%.

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 (RT) and at 37°C, and the human sE-selectin level determined after 24 h.
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.
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 used in one study.

13.7 Specificity

The assay detects both natural and recombinant human sE-selectin. The interference of circulating factors of the immune syteme was evaluated by spiking these proteins at physiologically relevant concentrations into serum.
There was no crossreactivity detected for IL-8, sICAM-1, sTNF-R, TNF-ß, CD8, IL-2R, IL-6 , L-selectin and P-selectin.

13.8 Expected Values

A panel of 28 sera samples from randomly selected apparently healthy donors (males and females) was tested for human sE-selectin.
The detected human sE-selectin levels ranged between 23.0 and 79.2 /ml with a mean level of 52.8 ng/ml and a standard deviation of 17.4 ng/ml.

14 Ordering Information

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* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20 x (25 ml) to 475 ml distilled water

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 130 µl distilled water to sample wells.
- Add 20 µl sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) on a microplate shaker at 400 rpm. Shaking is absolutely necessary for an optimal test performance.
- Empty and wash microwell strips 3 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 100 µl Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

Note: Samples have been diluted 1:5, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 5).