invitrogen

Human sL-selectin Coated ELISA Kit

PRODUCT INFORMATION & MANUAL

Enzyme-linked Immunosorbent Assay for quantitative detection of human sL-selectin

Catalog Number REF BMS206CE, BMS206TENCE

Σ 96 TESTS

CE IVD

For in-vitro diagnostic use. Not for therapeutic procedures.

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TABLE OF CONTENTS

1. Intended Use

The human sL-selectin ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sL-selectin. **The human sL-selectin ELISA is for in vitro diagnostic use. Not for use in therapeutic procedures.**

2. Summary

Leukocyte-Endothelial Cell Adhesion Molecule-1, L-selectin (LECAM-1, MEL-14, LAM-1, LEU-8, TQ1, LEC.CAM-1, DREG.56) belongs to the selectin family of adhesion molecule. Together with ELAM-1 (E-selectin) and GMP-140 (P-selectin) sL-selectin mediates the initial interactions of leukocytes 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 L-selectin, by 2 short consensus repeats similar to the short consensus units in complement regulatory proteins. 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. L-selectin in this aspect mediates rolling of PMN's on endothelial cells. The potential binding partners of L-selectin carry a negative charge, probably a sialic acid and/or sulphate, and may contain mannose and fucose. In addition, L-selectin may also interact with ELAM-1 which is expressed on cytokine-activated endothelial cells. L-selectin is constitutively expressed on most leukocytes (PMN's, monocytes, lymphocyte subsets) in a seemingly functional form. It is required for the binding of lymphocytes to the high endothelial venules of peripheral lymph nodes (and therefore serves as a lymphocyte recirculating receptor) and for the invasion of neutrophils into sites of inflammation. When neutrophils are activated, L-selectin is shed by proteolytic cleveage near the transmembrane span. Lymphocytes and monocytes can also shed L-selectin upon activation although the kinetics are significantly lower. A broad range of activating agents including C5a, fMLP, TNF, GM-CSF, IL-8 are effective in inducing this response. The shed form of sL-selectin (sL-selectin) is functionally active and at high concentrations can inhibit leukocyte attachment to endothelium. The main source for sL-selectin in serum seems to be tissue localized leukocytes.

Determination of soluble/circulating L-selectin could provide more detailed insights into the pathological modifications during various diseases:

- **allergy:** L-selectin expression is down-modulated on eosinophils recovered from bronchoalveolar lavage fluid after allergen provocation.
- **bronchoalveolar lavage (BAL):** BAL transiently promotes PMN/monocyte activation and recruitment to the bronchoalveolar space. The cells respond with a complete shedding of L-selectin when they extravasate from the blood into the bronchoalveolar space.
- **deep venous thrombosis (DVT):** A case can be made for the participation of PMN's in the initiation and propagation of venous thrombosis. Probably via L-selectin leukocytes adhere to areas of veins that serve as sites for initiation of thrombi.
- **HIV:** patients suffering from HIV-infection showed markedly elevated levels of sL-selectin in serum.
- **insulin-dependent diabetes mellitus (IDDM):** serum levels of L- selectin were found to be elevated in IDDM patients and in subjects at risk for developing IDDM.
- **Kawasaki Syndrome:** sL-selectin levels seem to be less than those of normals.
- **malignant B-cell populations:** B-cell chronic lymphocytic leukaemia, hairy cell leukaemia and mantle zone lymphoma are L- selectin positive.
- **neonatal bacterial infection:** in case of intra-uterine infection lymphocytes obtained from cord blood have a diminished L-selectin expression. This is independent of gestational age, birth weight, umbilical artery pH, hematocrit, leukocyte count, absolute neutrophil count, CRP-level or maternal fever.
- **sepsis:** patients suffering from sepsis showed markedly elevated levels of sL-selectin in serum. Vascular endothelial injury observed in overwhelming sepsis may be caused by neutrophil-derived enzymes. Adherence to

endothelium is a prerequisite for this process. Measurement of sL-selectin may provide further insights into the interrelationship between neutrophil activation and endothelial damage in gram-negative sepsis.

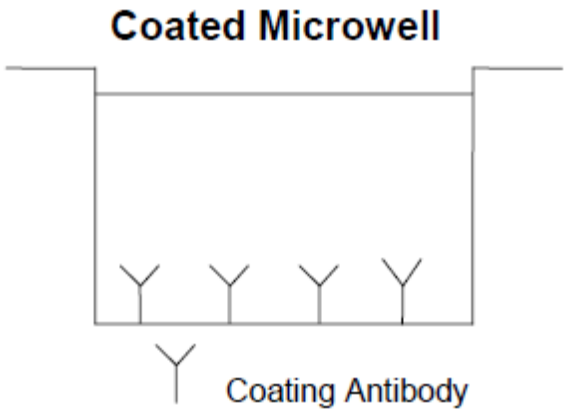
- **surgery:** patients undergoing cardiopulmonary bypass surgery may develop an acute post-operative capillary leak, due to endothelial injury inflicted by adherent neutrophils. In those patients L-selectin is completely lost in a small but progressively increasing proportion of PMN's, which could be responsible for the endothelial damage.

For literature update refer to **www.Thermo Fisher.com.**

3. Principles of the Test

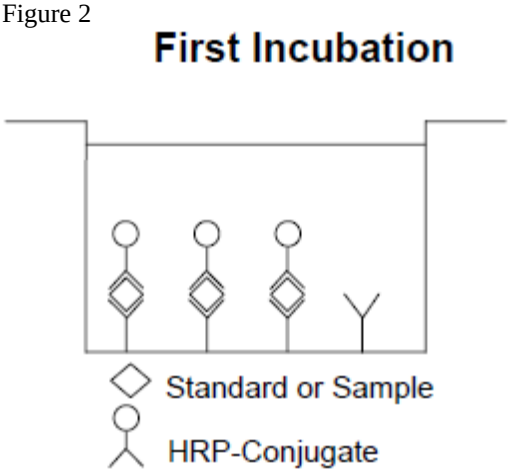
An anti-human sL-selectin coating antibody is adsorbed onto microwells.

Figure 1



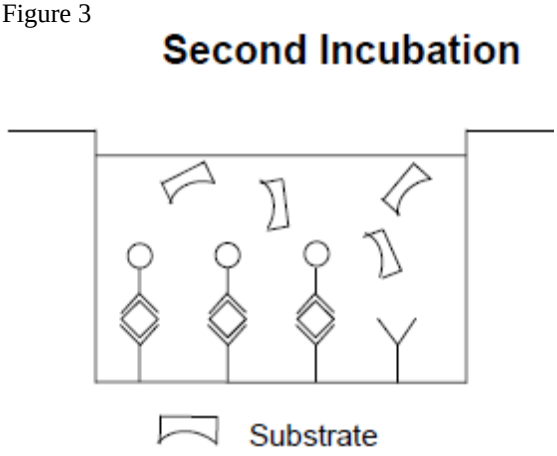
Human sL-selectin present in the sample or standard binds to antibodies adsorbed to the microwells and the HRP-conjugated anti- human sL-selectin antibody is added and binds to human sL-selectin captured by the first antibody.

Figure 2



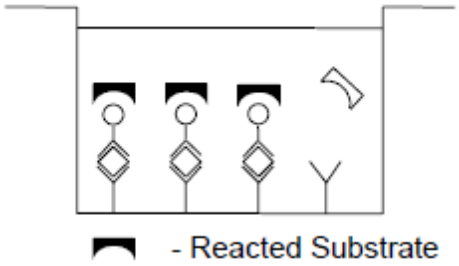
Following incubation unbound HRP- conjugated anti-human sL-selectin is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 3



A coloured product is formed in proportion to the amount of human sL-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 7 human sL- selectin standard dilutions and human sL- selectin concentration determined.

Figure 4



4. Reagents Provided

4.1 Reagents for human sL-selectin ELISA BMS206CE (96 tests)

1 aluminum pouch with a **Microwell Plate coated** with monoclonal antibody to human sL-selectin

1 vial (6 ml) **HRP-Conjugate** anti-human sL-selectin monoclonal antibody, ready to use

2 vials human sL-selectin **Standard** lyophilized, 50 ng/ml upon reconstitution

1 bottle (50 ml) **Sample Diluent**

1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)

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

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

2 **Adhesive Films**

4.2 Reagents for human sL-selectin ELISA BMS206TENCE (10x96 tests)

10 aluminum pouches with a **Microwell Plate coated** with monoclonal antibody to human sL-selectin

10 vials (6 ml) **HRP-Conjugate** anti-human sL-selectin monoclonal antibody, ready to use

10 vials human sL-selectin **Standard** lyophilized, 50 ng/ml upon reconstitution

10 bottles (50 ml) **Sample Diluent**

3 bottles (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)

10 vials (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (100 ml) **Stop Solution** (1M Phosphoric acid)

10 **Adhesive Films**

5. Storage Instructions – ELISA Kit

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). 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 (EDTA, 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.

Pay attention to a possible **"Hook Effect"** due to high sample concentrations (see chapter 11).

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human sL-selectin. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13.5).

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, 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 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 statement(s) for specific advice.
- Reagents are intended for in vitro diagnostic use and are not for use in 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 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 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

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

9.1. Wash Buffer (1x)

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.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

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. Human sL-selectin Standard

Reconstitute **human sL-selectin standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 50 ng/ml).
Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

After usage remaining standard cannot be stored and has to be discarded.

Standard dilutions can be prepared directly on the microwell plate (see 10.d) or alternatively in tubes (see 9.2.1).

9.2.1. External Standard Dilution

Label 7 tubes, one for each standard point.

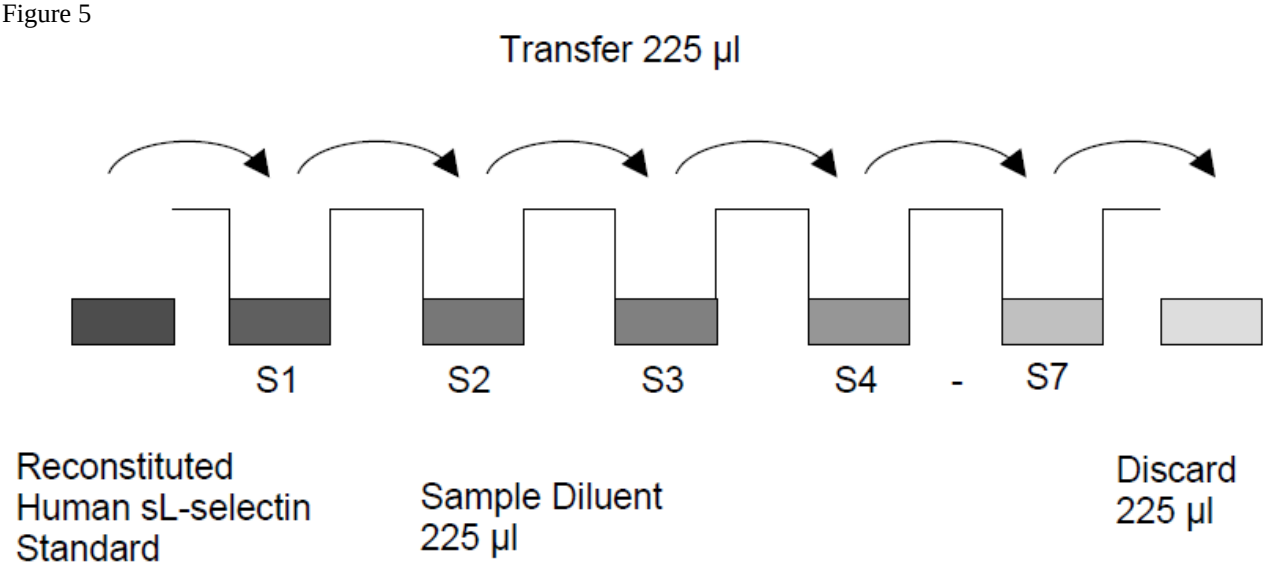
S1, S2, S3, S4, S5, S6, S7

Then prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µl of Sample Diluent into each tube.

Pipette 225 µl of reconstituted (concentration of standard = 50 ng/ml) into the first tube, labeled S1, and mix (concentration of standard 1 = 25 ng/ml).
Pipette 225 µl of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 5).

Sample Diluent serves as blank.

Figure 5



10. Test Protocol

1. Predilute your samples before starting with the test procedure. Dilute serum, plasma and cell culture samples 1:100 with Sample Diluent according to the following scheme:

   Dilution 1: 10 µl sample + 90 µl Sample Diluent

   Dilution 2: 50 µl of dilution 1 + 450 µl Sample Diluent

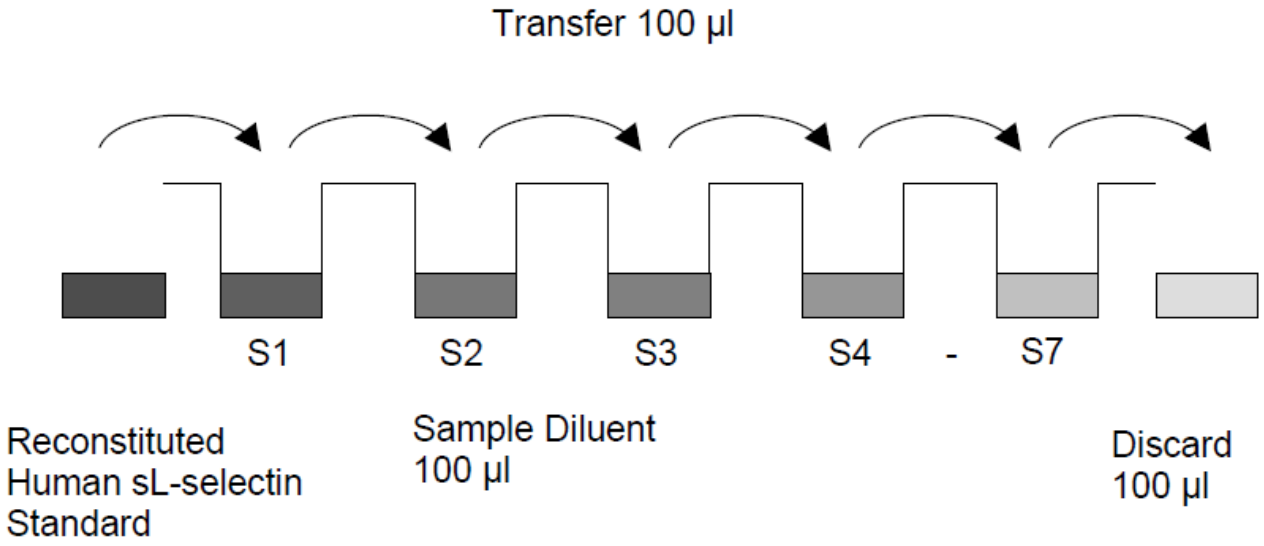
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

3. Wash the microwell strips twice 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 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**.

4. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 9.2.1): Add 100 µl of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.2, concentration = 50.0 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 = 25.0 ng/ml), and transfer 100 µl to wells B1 and B2, respectively (see Figure 6). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human sL-selectin standard dilutions ranging from 50.0 to 0.4 ng/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 6



In case of an **external standard dilution** (see 9.2.1), pipette 100 µl of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

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 (25.0 ng/ml)	Standard 1 (25.0 ng/ml)	Sample 1	Sample 1
B	Standard 2 (12.5 ng/ml)	Standard 2 (12.5 ng/ml)	Sample 2	Sample 2
C	Standard 3 (6.3 ng/ml)	Standard 3 (6.3 ng/ml)	Sample 3	Sample 3
D	Standard 4 (3.2 ng/ml)	Standard 4 (3.2 ng/ml)	Sample 4	Sample 4
E	Standard 5 (1.6 ng/ml)	Standard 5 (1.6 ng/ml)	Sample 5	Sample 5
F	Standard 6 (0.8 ng/ml)	Standard 6 (0.8 ng/ml)	Sample 6	Sample 6
G	Standard 7 (0.4 ng/ml)	Standard 7 (0.4 ng/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

5. Add 100 µl of **Sample Diluent** in duplicate to the **blank wells**.
6. Add 50 µl of Sample Diluent to the sample wells.
7. Add 50 µl of each **sample** in duplicate to the **sample wells**.
8. Add 50 µl of **HRP-Conjugate** to all wells.
9. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 400 rpm.
10. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.
11. Pipette 100 µl of **TMB Substrate Solution** to all wells.
12. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

    The color 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 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 µl 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 - 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.

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

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 sL-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 sL-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 sL-selectin concentration.
- **If instructions in this protocol have been followed, samples have been diluted 1:200 (1:100 external predilution, 1:2 dilution on the plate: 50 µl sample + 50 µl Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 200).**
- **Calculation of 1:100 prediluted samples with a concentration exceeding standard 1 may result in incorrect, low human sL- selectin levels (Hook Effect). Such samples require further external predilution according to expected human sL-selectin values with Sample Diluent in order to precisely quantitate the actual human sL-selectin level.**
- It is suggested that each testing facility establishes a control sample of known human sL-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. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 7
Representative standard curve for human sL-selectin ELISA. Human sL- selectin was diluted in serial 2-fold steps in Sample Diluent . Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

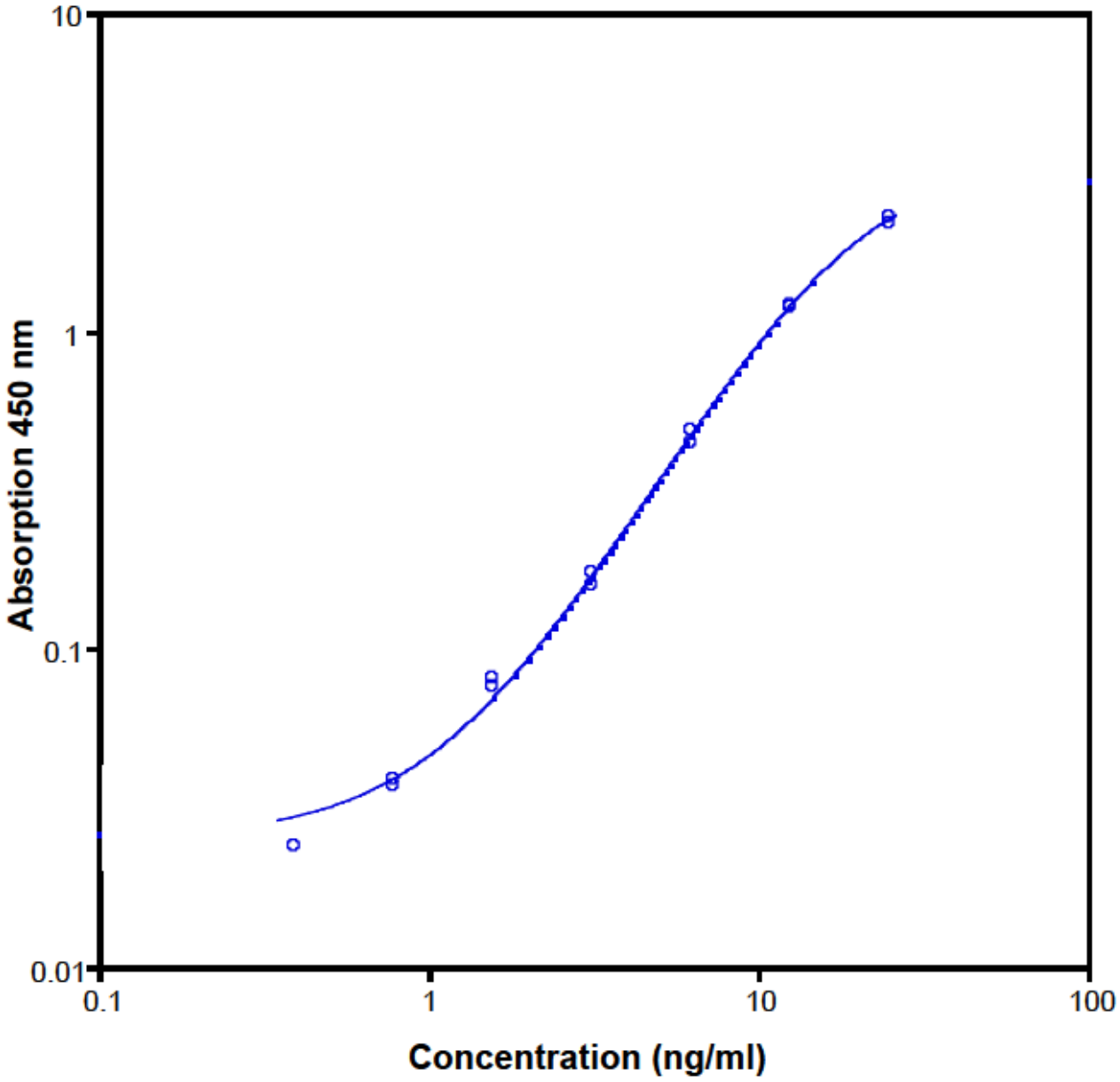


Table 2
Typical data using the human sL-selectin ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human sL-selectin Concentration (ng/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	25.0	2.273	2.228	2.0
		2.183		
2	12.5	1.207	1.189	1.5
		1.172		
3	6.3	0.489	0.465	
		0.440		
4	3.2	0.174	0.166	4.9
		0.158		
5	1.6	0.081	0.078	2.9
		0.076		
6	0.8	0.039	0.038	2.0
		0.037		
7	0.4	0.024	0.024	0
		0.024		
Blank	0	0.015	0.015	3.4
		0.014		

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

- 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 sL-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 0.198 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 sL-selectin. 2 standard curves were run on each plate. Data below show the mean human sL-selectin concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 3.7%.

Table 3

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

Sample	Experiment	Mean Human sL-selectin Concentration (ng/ml)	Coefficient of Variation (%)
1	1	1195.3	3.4
	2	1205.7	1.8
	3	1019.7	11.7
2	1	1144.3	1.8
	2	1162.8	2.8
	3	1002.9	2.5
3	1	1411.5	3.3
	2	1407.8	5.8
	3	1245.0	3.6
4	1	722.0	0.5
	2	774.2	2.1
	3	777.8	4.3
5	1	1301.6	1.7
	2	1284.5	6.6
	3	1216.8	6.0
6	1	1021.0	2.1
	2	997.7	2.9
	3	1010.2	6.0
7	1	867.4	2.0
	2	839.2	0.6
	3	808.4	4.3
8	1	603.1	2.3
	2	546.6	2.6
	3	560.0	7.4

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 sL- selectin. 2 standard curves were run on each plate. Data below show the mean human sL-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 4.2%.

Table 4

The mean human sL-selectin concentration and the coefficient of variation of each sample

Sample	Mean Human sL-selectin Concentration (ng/ml)	Coefficient of Variation (%)
1	1140.2	7.5
2	1103.3	6.5
3	1354.7	5.7
4	758.0	3.4
5	1267.6	2.9
6	1009.7	0.9
7	838.6	2.9
8	569.9	4.2

13.3. Spike Recovery

The spike recovery was evaluated by spiking 3 levels of human sL- selectin into serum. Recoveries were determined in 3 independent experiments with 6 replicates each.
The amount of endogenous human sL-selectin in unspiked serum was subtracted from the spike values.
The recovery ranged from 85% to 118% with an overall mean recovery of 99% (see Table 5).

Table 5

Experiment	Spike high (%)	Spike medium (%)	Spike low (%)
1	85	118	101
2	101	109	99
3	80	111	91

13.4. Dilution Parallelism

Serum samples with different levels of human sL-selectin were analysed at serial 2 fold dilutions with 4 replicates each.
The recovery ranged from 82% to 96% with an overall recovery of 89% (see Table 6).

Table 6

Sample	Dilution	Expected Human sL-selectin Concentration (ng/ml)	Observed Human sL-selectin Concentration (ng/ml)	Recovery of Expected human sL-selectin Concentration (%)
1	1:200	--	1498.4	--
	1:400	749.2	656.4	87.6
	1:800	374.6	351.2	93.8
	1:1600	187.3	154.0	82.2
2	1:200	--	1362.8	--
	1:400	681.4	613.8	90.1
	1:800	340.7	318.8	93.6
	1:1600	170.3	148.6	87.2
3	1:200	--	1576.0	--
	1:400	788.0	689.6	87.5
	1:800	394.0	374.0	94.9
	1:1600	197.0	166.3	84.4
4	1:200	--	957.9	--
	1:400	478.9	457.6	95.6
	1:800	239.5	202.2	84.4
	1:1600	119.7	104.6	87.4

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 sL-selectin levels determined. There was no significant loss of human sL-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 sL-selectin level determined after 24 h. There was no significant loss of human sL- selectin immunoreactivity detected during storage under above conditions.

13.6. Specificity

The assay detects both natural and recombinant human sL-selectin. The interference of IL-8, ICAM-1, TNF-R, TNF alpha, TNF beta, CD8, IL- 2, IL-2R, IL-6, IL-6R, IL-10, and E-selectin, CD44 and HER-2 was evaluated by spiking these proteins at physiologically relevant concentrations into a human sL-selectin positive serum.
There was no crossreactivity detected.

13.7. Expected Values

A panel of 22 sera samples from randomly selected apparently healthy donors (males and females) was tested for human sL-selectin.
The detected human sL-selectin levels ranged between 487.3 and 1096.3 ng/ml with a mean level of 842.0 ng/ml and a standard deviation of 168.9 ng/ml.

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. Human sL-selectin Standard

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

15. Test Protocol Summary

1. Predilute sample with Sample Diluent 1:100.
2. Determine the number of microwell strips required.
3. Wash microwell strips twice with Wash Buffer.
4. Standard dilution on the microwell plate: Add 100 µl Sample Diluent , in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells.

   Alternatively external standard dilution in tubes (see 9.2.1): Pipette 100 µl of these standard dilutions in the microwell strips.
5. Add 100 µl Sample Diluent , in duplicate, to the blank wells.
6. Add 50 µl Sample Diluent to sample wells.
7. Add 50 µl sample in duplicate, to designated sample wells.
8. Add 50 HRP-Conjugate, ready to use to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
10. Empty and wash microwell strips 3 times with Wash Buffer.

11. Add 100 µl of TMB Substrate Solution to all wells.
12. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
13. Add 100 µl Stop Solution to all wells.
14. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions in this protocol have been followed, samples have been diluted 1:200 (50 µl 1:100 prediluted sample + 50 µl Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 200).

PRODUKTINFORMATION UND HANDBUCH (Deutsch)

1. Mitgelieferte Reagenzien

1.1. Mitgelieferte Reagenzien für human sL-selectin ELISA **BMS206CE** (96 Tests)

1 Aluminiumbeutel mit **Mikrotiterplatte, beschichtet** mit Antikörper (monoklonal) gegen human sL-selectin

1 Fläschchen (6 ml) **HRP-Konjugat,** gebrauchsfertig, monoklonaler anti-human sL-selectin Antikörper

2 Fläschchen human sL-selectin-**Standard**, lyophilisiert, 50 ng/ml nach Rekonstitution

1 Flasche (50 ml) **Verdünnungslösung**

1 Flasche (50 ml) **Waschpufferkonzentrat** 20x (PBS mit 1% Tween 20)

1 Fläschchen (15 ml) **Substratlösung** (Tetramethylbenzidin)

1 Fläschchen (15 ml) **Stopplösung** (1 M Phosphorsäure)

2 **Klebefolien**

1.2. Mitgelieferte Reagenzien für human sL-selectin ELISA **BMS206TENCE** (10x96 Tests)

10 Aluminiumbeutel mit **Mikrotiterplatte, beschichtet** mit Antikörper (monoklonal) gegen human sL-selectin

10 Fläschchen (6 ml) **HRP-Konjugat,** gebrauchsfertig, monoklonaler anti-human sL-selectin Antikörper

10 Fläschchen human sL-selectin-**Standard**, lyophilisiert, 50 ng/ml nach Rekonstitution

10 Flaschen (50 ml) **Verdünnungslösung**

3 Flaschen (50 ml) **Waschpufferkonzentrat** 20x (PBS mit 1% Tween 20)

10 Fläschchen (15 ml) **Substratlösung** (Tetramethylbenzidin)

1 Fläschchen (100 ml) **Stopplösung** (1 M Phosphorsäure)

10 **Klebefolien**

2. Lagerhinweise

Lagern Sie den Inhalt des Kits bei 2°-8°C. Verbliebene Reagenzien nach Verwendung sofort wieder auf 2°-8°C kühlen. Das Ablaufdatum des Kits und der Reagenzien ist auf den Etiketten angegeben.
Die Haltbarkeit des Kits und der Komponenten kann nur bei fachgerechter Lagerung garantiert werden, sowie bei mehrfacher Verwendung nur dann, wenn die Reagenzien bei der ersten Verwendung nicht kontaminiert wurden.

3. Sicherheitsvorkehrungen für den Gebrauch

- Alle enthaltenen Reagenzien sollten als potenziell gefährlich betrachtet werden. Daher wird empfohlen, dass dieses Produkt nur von Personen mit labortechnischer Erfahrung und in Übereinstimmung mit GLP Richtlinien verwendet wird. Passende Schutzbekleidung, wie Labormäntel, Sicherheitsbrillen und Laborhandschuhe müssen getragen werden. Vermeiden Sie jeden Kontakt der Reagenzien mit Haut oder Augen. Im Falle des Kontaktes von Reagenzien mit Haut oder Augen, sofort mit Wasser spülen. Bitte entnehmen Sie weitere spezifische Hinweise den Sicherheitsdatenblättern und/oder den Sicherheitsbestimmungen.
- Die Reagenzien sind ausschließlich für Diagnosezwecke bestimmt und nicht für den Einsatz bei Therapien.
- Reagenzien aus verschiedenen Chargen oder anderer Herkunft nicht mischen oder untereinander austauschen.
- Verwenden Sie die Kitreagenzien nicht nach dem Ablaufdatum (siehe Etikett).
- Setzen Sie die Kitreagenzien während der Lagerung oder Inkubation keiner starken Lichteinstrahlung aus.
- Nicht mit dem Mund pipettieren.
- In Bereichen, in denen mit Kitreagenzien oder Proben hantiert wird, nicht essen, trinken oder rauchen.
- Vermeiden Sie den Kontakt der Haut/Schleimhäute mit Kitreagenzien/Proben.
- Tragen Sie während des Hantierens mit Kitreagenzien oder Proben geeignete Gummi- oder Einweghandschuhe.
- Vermeiden Sie den Kontakt zwischen Substratlösung und Oxidationsmitteln/Metallen.
- Vermeiden Sie Verspritzen von Flüssigkeit oder Bildung von Aerosolen.
- Zur Vermeidung von Kontamination mit Mikroben oder Kreuzkontamination der Reagenzien oder Proben, die den Test ungültig machen könnten, verwenden Sie Einwegpipettenspitzen und/oder Einwegpipetten.
- Verwenden Sie saubere, geeignete Reagenzgefäße für das Dispensieren von Konjugat und Substratreagenzien.
- Vermeiden Sie Kontakt mit Säuren, da dadurch Konjugate inaktiviert werden.
- Für die Reagensherstellung muss destilliertes oder entionisiertes Wasser verwendet werden.
- Die Substratlösung muss vor der Verwendung auf Raumtemperatur gebracht werden.
- Dekontaminieren und entsorgen Sie Proben sowie alle möglicherweise kontaminierten Materialien so, als ob sie Infektionserreger enthalten könnten. Die bevorzugte Dekontaminationsmethode ist Autoklavieren für mind. eine Stunde bei 121.5°C.
- Flüssige Abfälle, die kein Säure enthalten, sowie neutralisierte Abfälle werden zur Dekontamination mit Natrium Hypochlorit versetzt (Endkonzentration von Natrium Hypochlorit 1.0%). Nach 30 min ist eine effektive Dekontamination erreicht. Flüssige Abfälle, die Säure enthalten, müssen vor der Dekontamination neutralisiert werden.

4. Vorbereitung der Reagenzien

Bringen Sie das **Pufferkonzentrat** auf Raumtemperatur und stellen Sie Verdünnungen vor Beginn des Tests her. Sollten sich im **Pufferkonzentrat** Kristalle gebildet haben, erwärmen Sie diese vorsichtig bis zur vollständigen Auflösung der Kristalle.

4.1. Waschpuffer (1x)

Leeren Sie den gesamten Inhalt (50 ml) des **Waschpufferkonzentrats** (20x) in einen sauberen 1000-ml-Messzylinder. Füllen Sie mit destilliertem oder entionisiertem Wasser auf, bis ein Endvolumen von 1000 ml erreicht ist. Mischen Sie vorsichtig um Schäumen zu vermeiden.

Füllen Sie in eine saubere Waschflasche um und lagern Sie den Waschpuffer (1x) bei 2° bis 25°C lagern. Bitte beachten Sie, dass dieser 30 Tage haltbar ist.

Der benötigte Waschpuffer (1x) kann auch entsprechend der untenstehenden Tabelle hergestellt werden:

Anzahl der Streifen	Waschpufferkonzentrat (20x) (ml)	Destilliertes Wasser (ml)
1 - 6	25	475
1 - 12	50	950

4.2. Human sL-selectin-Standard

Rekonstituieren Sie den human sL-selectin standard durch Zugabe von destilliertem Wasser. Das Rekonstitutionsvolumen ist auf dem Standardfläschchen angegeben. Rühren oder mischen Sie vorsichtig um eine vollständige und homogene Auflösung zu erzielen (Konzentration des rekonstituierten Standards = 50 ng/ml).
Den rekonstituierten Standard nach 10-30 min verdünnen und davor gut mischen.

Der Standard muß sofort nach Rekonstitution verwendet und kann nicht gelagert werden.

Die **Standardverdünnungen** können direkt auf den Mikrotiterplatten (siehe 5.d) oder in Reaktionsgefäßen (siehe 4.2.1) hergestellt werden.

4.2.1. Externe Standardverdünnung

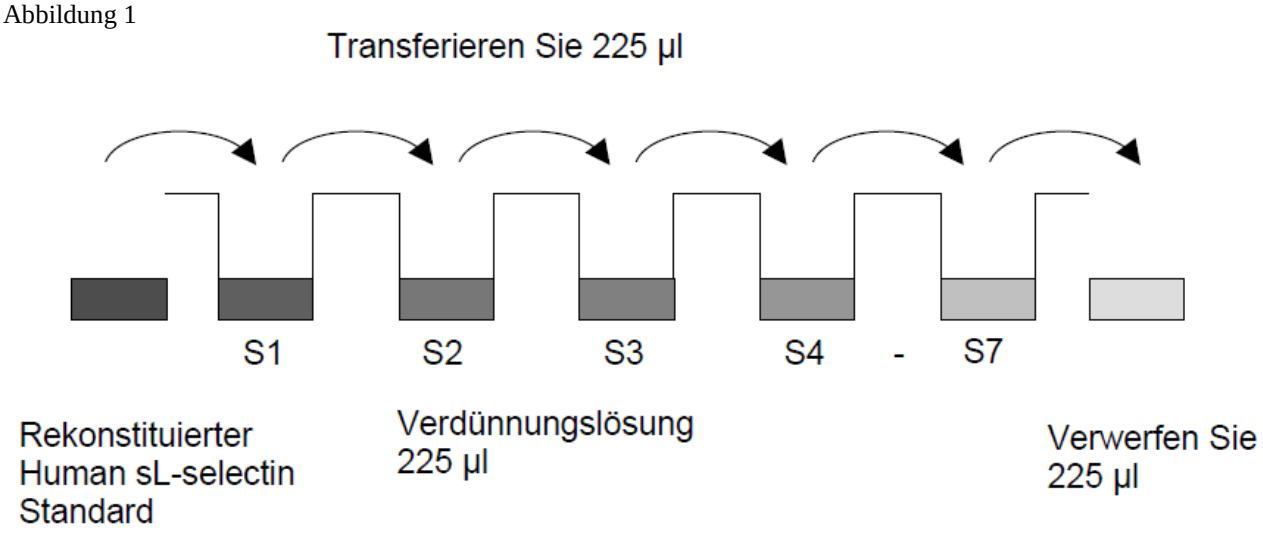
Beschriften Sie 7 Gefäße, jedes für einen Standardpunkt.: S1, S2, S3, S4, S5, S6, S7

Danach stellen Sie eine 1:2 Verdünnungsreihe für die Standardkurve her:
Pipettieren Sie in jedes Gefäß 225 µl der Verdünnungslösung.

Pipettieren Sie 225 µl des rekonstituierten Standards (Konzentration des Standards = 50 ng/ml) in das erste Gefäß mit der Beschriftung S1 und mischen Sie (Konzentration des Standard 1 = 25 ng/ml).
Pipettieren Sie 225µl dieser Verdünnung in das zweite Gefäß (mit der Beschriftung S2) und mischen Sie sorgfältig vor dem nächsten Verdünnungsschritt.
Wiederholen Sie diese Verdünnungsschritte 5x. Die so hergestellte Verdünnungsreihe dient zur Erstellung der Standardkurve (siehe Abbildung 1).

Verdünnungslösung dient als Blindwert.

Abbildung 1



5. Testprotokoll

1. Verdünnen Sie ihre Proben bevor Sie den Test beginnen: Verdünnen Sie Serum,Plasma und Zellkultur Proben 1:100 wie folgt mit Verdünnungslösung

   I) 10 µl Probe + 90 µl Verdünnungslösung

   II) 50 µl vorverdünnte Probe + 450 µl Verdünnungslösung

2. Bestimmen Sie die Anzahl der Mikrowellstreifen die für das Testen der gewünschten Anzahl von Proben benötigt werden, sowie die Mikrowellstreifen für Blindwert und Standards. Probe, Standard und Blindwert immer jeweils doppelt testen. Entfernen Sie die zusätzlichen Mikrowellstreifen von der Halterung und bewahren Sie diese mit dem mitgelieferten Trockenmittel in dem Folienbeutel fest verschlossen bei 2°-8°C auf.

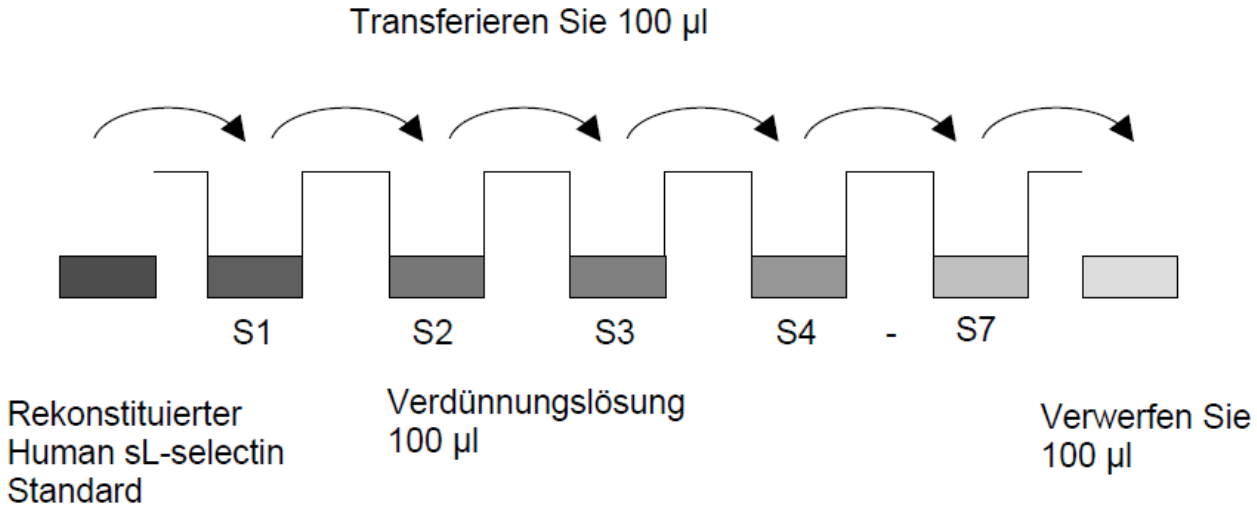
3. Waschen Sie die Mikrowellstreifen 2 mal mit ca. 400 µl **Waschpuffer** pro Vertiefung; zwischen den Waschgängen den Inhalt der Vertiefungen gründlich absaugen. Vor dem Absaugen Waschpuffer **10-15 Sekunden** einwirken lassen. Achten Sie darauf, die Oberfläche der Vertiefungen nicht zu zerkratzen.

   Leeren Sie die Vertiefungen nach dem letzten Waschschritt und klopfen Sie die Mikrowellstreifen auf einem Saug- oder Papiertuch aus um überschüssigen Waschpuffer zu entfernen. Verwenden Sie die Mikrowellstreifen sofort nach dem Waschen, oder legen Sie diese für maximal 15 min umgedreht auf ein nasses Saugtuch. **Lassen Sie die Vertiefungen nicht austrocknen.**

4. **Standardverdünnung auf der Mikrotiterplatte** (Wahlweise können die Standardverdünnungen auch in Reaktionsgefäßen hergestellt werden – siehe 4.2.1)

   Pipettieren Sie 100 µl Verdünnungslösung in alle **Standardvertiefungen**. Pipettieren Sie 100 µl des rekonstituierten 1: **Standards** (siehe Herstellung des Standards 4.2.1, Konzentration des Standards = 50.0 ng/ml) in die Vertiefungen A1 und A2 (Doppelbestimmung, siehe Tabelle 1). Mischen Sie den Inhalt der Vertiefungen A1 und A2 durch wiederholtes Aufsaugen und Zugeben gut durch (Konzentration des Standards S1 = 25.0 ng/ml) und transferieren Sie 100 µl in die Probenvertiefungen B1 und B2 (siehe Abbildung 2). Achten Sie darauf, die Oberfläche der Vertiefungen nicht zu zerkratzen. Wiederholen Sie diese Verdünnungsschritte 5 x, wodurch zwei human sL-selectin Verdünnungsreihen mit den Konzentrationen von 50.0 bis 0.4 ng/ml hergestellt werden. Verwerfen Sie 100 µl aus den letzten Standardvertiefungen (G1/2). Die so hergestellten Verdünnungsreihen

Abbildung 2



Falls sie eine **externe Standardverdünnungsreihe** erstellen (siehe 4.2.1), pipettieren Sie 100 µl der Standardverdünnungen (S1 – S7) in die Standardvertiefungen (entsprechend Tabelle 1).

Tabelle 1

Diagramm mit Beispiel für die Anordnung von Blindwert, Standards und Proben in den Mikrowellstreifen:

	1	2	3	4
A	Standard 1 (25.0 ng/ml)	Standard 1 (25.0 ng/ml)	Probe 1	Probe 1
B	Standard 2 (12.5 ng/ml)	Standard 2 (12.5 ng/ml)	Probe 2	Probe 2
C	Standard 3 (6.3 ng/ml)	Standard 3 (6.3 ng/ml)	Probe 3	Probe 3
D	Standard 4 (3.2 ng/ml)	Standard 4 (3.2 ng/ml)	Probe 4	Probe 4
E	Standard 5 (1.6 ng/ml)	Standard 5 (1.6 ng/ml)	Probe 5	Probe 5
F	Standard 6 (0.8 ng/ml)	Standard 6 (0.8 ng/ml)	Probe 6	Probe 6
G	Standard 7 (0.4 ng/ml)	Standard 7 (0.4 ng/ml)	Probe 7	Probe 7
H	Blindwert	Blindwert	Probe 8	Probe 8

5. Pipettieren Sie in alle **Blindwertvertiefungen** (Doppelbestimmung), 100 µl **Verdünnungslösung.**
6. Pipettieren Sie in alle **Probenvertiefungen** 50 µl Verdünnungslösung.
7. Pipettieren Sie je 50 µl von jeder **Probe** (Doppelbestimmung) in die **Probenvertiefungen** und mischen Sie den Inhalt durch.
8. Pipettieren Sie in alle Vertiefungen, einschließlich der Blindwertvertiefungen 50 µl **HRP-Konjugat.**
9. Mit einer Klebefolie abdecken und bei Raumtemperatur (18° bis 25°C) für 2 Stunden inkubieren, wenn möglich auf einem Schüttler bei 400 U/min.
10. Entfernen Sie die Klebefolie und entleeren Sie die Vertiefungen. **Waschen** Sie die Mikrowellstreifen 3 mal wie in Punkt c des Testprotokolls beschrieben. Verwenden Sie die Mikrowellstreifen sofort nach dem Waschen.
11. Pipettieren Sie in alle Vertiefungen, einschließlich der Blindwertvertiefungen, 100 µl **TMB-Substratlösung**.
12. Inkubieren Sie die Mikrowellstreifen bei Raumtemperatur (18° bis 25°C) für ca. 10 Minuten. Vermeiden Sie direkte, starke Lichteinstrahlung.

    Die Farbentwicklung innerhalb der einzelnen Vertiefungen muss beobachtet und die Substratreaktion gestoppt werden (siehe nächster Protokollpunkt), bevor die gefärbten Vertiefungen nicht mehr richtig gemessen werden können.
    Die optimale Inkubationszeit für die Farbentwicklung muß bei jedem Versuch neu bestimmt werden.

    Es wird empfohlen, die Stopplösung zuzugeben, wenn der höchste Standardpunkt eine dunkelblaue Farbe angenommen hat. Alternativ kann die Farbentwicklung auch mit einem Photometer bei 620 nm verfolgt werden. Die Substratreaktion sollte gestoppt werden, wenn der höchste Standardpunkt eine OD von 0.9 -0.95 erreicht.
13. Stoppen Sie die Enzymreaktion durch rasche Zugabe von 100 µl **Stopplösung** in jede Vertiefung, einschließlich der Blindwertvertiefungen. Für eine vollständige Inaktivierung der Enzyme ist es wichtig, die Stopplösung rasch und gleichmäßig in den Vertiefungen zu verteilen. Die OD Werte müssen sofort nach Beigabe der Stopplösung oder innerhalb einer Stunde nach Lagerung der Mikrowellstreifen in Dunkelheit bei 2-8°C gemessen werden.

14. Messen Sie die Absorption jeder Vertiefung mit einem Spektrophotometer. Verwenden Sie dabei 450 nm als primäre Wellenlänge (optional 620 nm als Referenzwellenlänge; 610 nm bis 650 nm sind möglich). Stellen Sie das Plattenmessgerät nach Anleitung des Herstellers und unter Verwendung der Blindwertvertiefungen auf den Leerwert ein. Bestimmen Sie die Absorption der Proben wie auch der human sL-selectin-Standards.

Die Proben wurden im Zuge der Testdurchführung 1:200 verdünnt. Daher muß der aus der Standardkurve berechnete Wert mit dem Verdünnungsfaktor multipliziert werden (x 200).

Anmerkung: Falls die Platte während der Inkubation nicht geschüttelt wurde, können die erreichten OD Werte niedriger als die unten angeführten sein. Die Ergebnisse sind trotzdem gültig.

INFORMACIÓN Y MANUAL DEL PRODUCTO (Espanol)

1. Reactivos Suministrados

1.1. Reactivos Suministrados para human sL-selectin ELISA **BMS206CE** (96 tests)

1 bolsa de aluminio con **una placa de micropocillos recubiertos** con anticuerpos monoclonales anti-human sL-selectin

1 vial (6 ml) con **conjugado de HRP,** listo para utilizar (anticuerpos monoclonales anti-human sL-selectin)

2 viales con **Estándar** human sL-selectin liofilizado, 50 ng/ml tras la reconstitución

1 frasco (50 ml) de **diluyente de muestra**

1 frasco (50 ml) de **concentrado de tampón de lavado** 20x (PBS con Tween 20 al 1%)

1 vial (15 ml) de **solución de sustrato** (tetrametil-bencidina)

1 vial (15 ml) de **solución de parada** (ácido fosfórico 1M)

2 **tapas para placas**, adhesives

1.2. Reactivos Suministrados para human sL-selectin ELISA **BMS206TENCE** (10x96 tests)

10 bolsas de aluminio con **una placa de micropocillos recubiertos** con anticuerpos monoclonales anti-human sL-selectin

10 viales (6 ml) con **conjugado de HRP,** listo para utilizar (anticuerpos monoclonales anti-human sL-selectin)

10 viales con **Estándar** human sL-selectin liofilizado, 50 ng/ml tras la reconstitución

10 frascos (50 ml) de **diluyente de muestra**

3 frascos (50 ml) de **concentrado de tampón de lavado** 20x (PBS con Tween 20 al 1%)

10 viales (15 ml) de **solución de sustrato** (tetrametil-bencidina)

1 vial (100 ml) de **solución de parada** (ácido fosfórico 1M)

10 **tapas para placas**, adhesives

2. Instrucciones de Conservación

Conservar los reactivos del kit a una temperatura comprendida entre 2 y 8ºC. Inmediatamente después de utilizarlos deberá volver a conservar los reactivos a dicha temperature (2° to 8°C). En las etiquetas figuran las fechas de caducidad del kit y de los reactivos.
Sólo se podrá garantizar la fecha de caducidad de los componentes del kit si se conservan adecuadamente y, en caso de uso reiterado de un mismo componente, si el reactivo no queda contaminado en la primera manipulación.

3. Precauciones de uso

- Todos los productos químicos deben considerarse potencialmente peligrosos. Por tanto, recomendamos que este producto sea manipulado únicamente por aquellas personas que hayan sido entrenadas en técnicas de laboratorio y que sea usado de acuerdo con los principios de buenas prácticas de laboratorio. Se debe llevar ropa de protección apropiada como puedan ser las batas de laboratorio, gafas de seguridad y guantes. Se debe trabajar con cuidado para evitar cualquier contacto con piel y ojos. En el caso de que tenga lugar un contacto con piel u ojos, proceder de forma inmediata a lavar la parte afectada con abundante agua. Véase la(s) hoja(s) de seguridad y/o declaraciones de seguridad para recomendaciones específicas.
- Los reactivos están destinados para un uso en diagnóstico in vitro y no se deben usar en procedimientos terapéuticos.
- No mezclar o sustituir los reactivos por los equivalentes de otros lotes u otras fuentes.
- No usar reactivos caducados.
- No exponer los reactivos del kit a una luz intensa durante su almacenamiento o incubación.
- No pipetear con la boca.
- No se recomienda comer o fumar en las zonas donde se manipulen muestras o reactivos.
- Evitar el contacto de los reactivos del kit o de las muestras con piel o mucosas.
- Se recomienda el uso de guantes desechables de goma o látex durante la manipulación de las muestras y reactivos.
- Evitar el contacto de la solución de sustrato con agentes oxidantes y metales.
- Evitar salpicaduras y la generación de aerosoles.
- Con el propósito de evitar una contaminación microbiológica o contaminaciones cruzadas de reactivos y muestras que puedan invalidar el test se recomienda el uso de pipetas y/o puntas de pipetas de un solo uso.
- Usar recipientes limpios y específicos de reactivos para la dispensación de reactivos de sustrato.
- La exposición a los ácidos inactiva el conjugado.
- Se debe usar agua destilada o desionizada en la preparación de los reactivos.
- La solución de sustrato debe de estar a temperatura ambiente antes de su uso.
- Descontaminar y disponer las muestras y todos los materiales potencialmente contaminados como si pudieran contener agentes infecciosos. El método preferente de descontaminación es un autoclavado durante un mínimo de 1 hora a 121.5ºC.
- Los residuos líquidos que no contengan ácido y los residuos neutralizados pueden ser mezclados con hipoclorito sódico en volúmenes tales que la mezcla final contenga 1.0% de hipoclorito sódico. Dejar actuar durante 30 minutos para una efectiva descontaminación. Los residuos líquidos que contengan ácido deben ser neutralizados previamente a la adición de hipoclorito sódico.

4. Preparación de los Reactivos

El **tampón concentrado** debe de alcanzar la temperatura ambiente y ser diluido antes de iniciar el procedimiento del test. Si en el concentrado de **tampón concentrado** se han formado cristales, caliente suavemente hasta su completa disolución.

4.1. Tampón de Lavado (1x)

Vierta todo el contenido (50 ml) del **concentrado de tampón de lavado** (20x) en un matraz aforado de 1000 ml limpio. Enrase en matraz con agua destilada o desionizada. Mezcle suavemente para evitar la formación de espuma.

Transfiera la solución a un frasco de lavado limpio y consérvela a una temperatura entre 2°C y 25°C. El tampón de lavado permanece estable durante 30 días.

En función de la cantidad que vaya a necesitar, prepare el tampón de lavado de acuerdo a la siguiente tabla:

Número de tiras	Tampón de lavado (20x) (ml)	Agua destilada (ml)
1 – 6	25	475
1 - 12	50	950

4.2. Dilución estándar human sL-selectin

Reconstituir el **estándar human sL-selectin** la adición de agua. El volumen de reconstitución está indicado en la etiqueta del vial del estándar. Girar o mezclar cuidadosamente para garantizar una completa y homogénea solubilización (concentración del estándar reconstituido = 50 ng/ml).
Permitir que el estándar reconstituido se asiente durante 10-30 minutos. Mezclar bien previamente a realizar las diluciones.

Tras su uso los restos del estándar no pueden ser almacenados y deben ser descartados.

Las **diluciones estándar** pueden ser preparadas directamente en la placa multipocillo (véase 5.d) o alternativamente en tubos (véase 4.2.1).

4.2.1. Dilución Estándar Externa

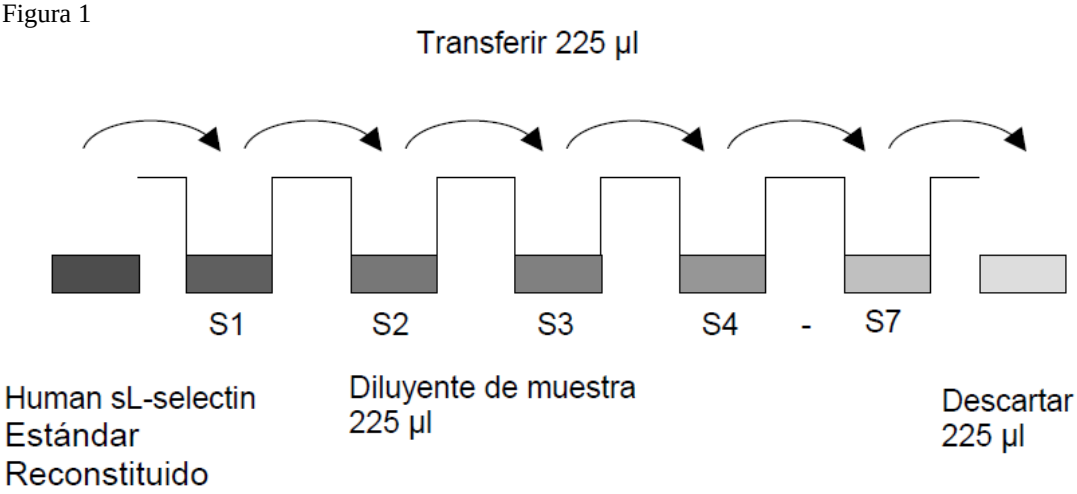
Rotular 7 tubos, uno para cada punto de la curva estándar.

S1, S2, S3, S4, S5, S6, S7.

Acto seguido, preparar diluciones seriadas 1:2 para la curva estándar como se indica a continuación:
Pipetear 225 µl de Diluyente de muestra a todos los tubos.
Pipetear 225 µl de estándar reconstituido (concentración del estándar = 50 ng/ml) en el primer tubo, etiquetado como S1, y mezclar (concentración del estándar 1 = 25 ng/ml).
Pipetear 225 µl de esta dilución en el segundo tubo, etiquetado como S2, y mezclar completamente antes de la siguiente transferencia. Repetir la serie de diluciones 5 veces más de manera que se obtengan los diferentes puntos de la curva estándar (véase Figura 1).

Diluyente de muestra sirve como blanco.

Figura 1



5. Protocolo de Ensayo

1. Diluya las muestras 1:100 con el Diluyente de muestra de acuerdo con el siguiente esquema:

   I) 10 µl de muestra + 90 µl de Diluyente de muestra

   II) 50 µl de muestra prediluida+ 450 µl de Diluyente de muestra

2. Determine el número de tiras necesarias para analizar el número deseado de muestras y además añada las tiras para blancos y patrones (de color). Todas las muestras, estándares y blancos deben ser analizadas por duplicado. Retire del soporte las tiras sobrantes y consérvelas, junto con el desecante suministrado en una bolsa metalizada y cerrada herméticamente, a una temperatura de 2°-8° C. Coloque las tiras que contienen la curva de valoración en las posiciones A1/A2 a H1/H2 (véase la Tabla 1).

3. Lave 2 veces las tiras con aproximadamente 400 µl de **tampón de lavado** por cada pocillo, aspirando completamente el contenido de los pocillos entre cada lavado. Permitir que el tampón de lavado permanezca en los pocillos durante **10-15 segundos** antes de su aspiración. Evite rayar la superficie de los pocillos.

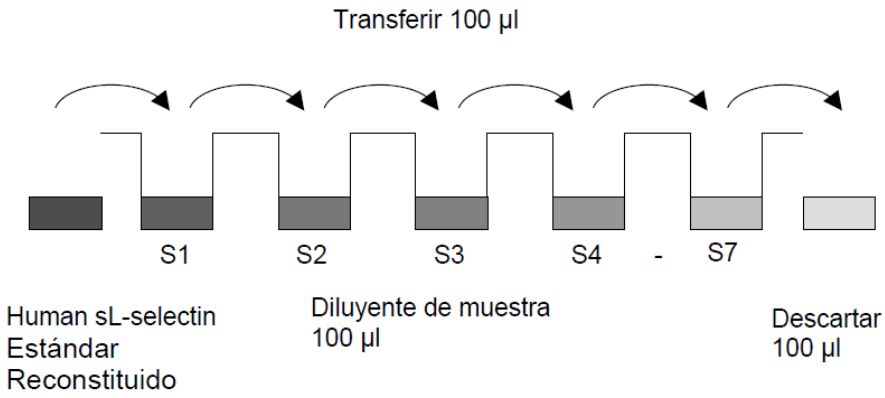
   Tras el último lavado, golpee suavemente las tiras contra un papel absorbente o una toallita de papel para eliminar el exceso de tampón de lavado. Utilice las tiras inmediatamente después de lavadas o bien colóquelas boca abajo sobre un papel absorbente húmedo durante como máximo 15 minutos. **No deje secar los pocillos.**

4. **Dilución de los Estándars en la placa multipocillo** (Alternativamente, la dilución de los estándars puede ser preparada en tubos – véase 4.2.1)

   Añadir 100 µl de Diluyente de muestra a todos los pocillos estándar. Pipetear 100 µl de estándar preparado (véase Preparación del Estándar 4.2, concentración = 50.0 ng/ml) por duplicado en los pocillos A1 y A2 (véase Tabla 1). Mezclar el contenido de los pocillos A1 y A2 por repetidas aspiraciones y expulsiones del contenido con la pipeta (concentración del estándar 1, S1 =25.0 ng/ml), y transferir 100 µl a los pocillos B1 y B2, respectivamente (véase Figura 2). Levar cuidado de no rascar la superficie interior de los micropocillos con la punta de la pipeta. Continuar este procedimiento 5 veces, formando dos filas de diluciones estándar del human sL-selectin ordenadas des de 25.0 a 0.4 ng/ml.

   Descartar 100 µl de los contenidos de los últimos micropocillos (G1, G2) usados.

Figura 2



En caso de **una dilución estándar externa** (véase 4.2.1), pipetear 100 µl de estas diluciones estándar (S1 - S7) en los pocillos correspondientes al estándar de acuerdo con la Tabla 1.

5. Añada 100 µl Diluyente de muestra a los pocillos del blanco, por duplicado.
6. Añada 50 µl de Diluyente de muestra a los pocillos con muestras.
7. Por duplicado, añada 50 µl de cada **muestra** a **los pocillos designados**.

Tabla 1

Tabla que describe un ejemplo de la disposición de los blancos, estándars y muestras en los micropocillos de las tiras:

	1	2	3	4
A	Estándar 1 (25.0 ng/ml)	Estándar 1 (25.0 ng/ml)	Muestra 1	Muestra 1
B	Estándar 2 (12.5 ng/ml)	Estándar 2 (12.5 ng/ml)	Muestra 2	Muestra 2
C	Estándar 3 (6.3 ng/ml)	Estándar 3 (6.3 ng/ml)	Muestra 3	Muestra 3
D	Estándar 4 (3.2 ng/ml)	Estándar 4 (3.2 ng/ml)	Muestra 4	Muestra 4
E	Estándar 5 (1.6 ng/ml)	Estándar 5 (1.6 ng/ml)	Muestra 5	Muestra 5
F	Estándar 6 (0.8 ng/ml)	Estándar 6 (0.8 ng/ml)	Muestra 6	Muestra 6
G	Estándar 7 (0.4 ng/ml)	Estándar 7 (0.4 ng/ml)	Muestra 7	Muestra 7
H	Blanco	Blanco	Muestra 8	Muestra 8

8. Añada 50 µl **conjugado de HRP** a todos los pocillos.
9. Cubra la placa con una tapa e incúbela a temperatura ambiente (18°C - 25°C) durante 2 horas (en un agitador mecánico a 100 rpm, si es posible).
10. Retire la tapa y vacíe los pocillos. **Lavar** los micropocillos de las tiras 3 veces de acuerdo al punto c del protocolo del test. Proseguir inmediatamente después al próximo paso.
11. Pipetee 100 µl de **solución de sustrato TMB** y viértalos en todos los pocillos, incluidos los del blanco.
12. Incube las tiras a temperatura ambiente (18°C - 25°C) durante aproximadamente 10 minutos. Evite la exposición directa a la luz intensa.

Deben monitorizarse los valores DO de la placa para detener la reacción del sustrato (véase el siguiente punto de este protocolo) antes de que deje de ser posible registrar correctamente los pocillos positivos.

La determinación del tiempo adecuado para el desarrollo del color, debe realizarse de forma individual para cada ensayo.

Se recomienda añadir la solución de parada cuando el estándar más alto presente un color azul oscuro. Alternativamente el desarrollo de color puede ser monitorizado con un lector de placas de ELISA a 620 nm. La reacción del substrato debería ser parada cuando este estándard alcanze una DO entre 0.9 y 0.9 5.

13. Detenga la reacción enzimática pipeteando rápidamente 100 µl de **solución de parada** en cada pocillo, incluidos los del blanco. Es importante dispensar la solución de parada de forma rápida y uniforme en todos los pocillos para inactivar totalmente la enzima. Los resultados deben leerse inmediatamente después de añadir la solución de parada o, como máximo, en el plazo de 1 hora si las tiras se conservan a una temperatura entre 2 - 8°C en un lugar oscuro.
14. Lea la absorbancia de cada pocillo en un espectrofotómetro utilizando 450 nm como longitud de onda principal (opcionalmente 620 nm como longitud de onda de referencia; los valores comprendidos entre 610 nm y 650 nm son aceptables). Utilizando los pocillos de blanco, haga el blanco del lector de placas de acuerdo con las instrucciones del fabricante. Determine la absorbancia de las muestras y de los human sL-selectin.

Las muestras han sido diluidas 1: 200, por tanto la concentración leída a partir de la curva estándar debe ser multiplicada por el factor de dilución (x 200).

Nota: En caso de incubar sin agitar, los valores de D.O. pueden ser inferiores a los indicados más abajo. De todas formas los resultados siguen siendo válidos.

INFORMATIONS SUR LE PRODUIT ET MANUEL (Français)

1. Réactifs Fournis

1.1. Reactifs pour ELISA de human sL-selectin BMS206CE (96 essais)

1 pochette en aluminium contenant **une plaque de microtitration** recouverte d'anticorps monoclonaux anti-human sL-selectin

1 flacon (6 ml) de **conjugué HRP** anti-human sL-selectin (anticorps monoclonaux anti-human sL-selectin)**,** prêt à utilizer

2 flacons d'**étalon** human sL-selectin, lyophilisé, 50 ng/ml après reconstitution

1 flacon (50 ml) **diluant d'échantillon**

1 flacon (50 ml) de **tampon de lavage concentré** 20 x (PBS avec du Tween 20 1 %)

1 flacon (15 ml) de **solution de substrat** (tétraméthyle-benzidine)

1 flacon (15 ml) de **solution d'arrêt** (acide phosphorique 1 M)

2 **couvre-plaques** adhésifs

1.2. Reactifs pour ELISA de human sL-selectin BMS206TENCE (10x96 essais)

10 pochettes en aluminium contenant **une plaque de microtitration** recouverte d'anticorps monoclonaux anti-human sL-selectin

10 flacons (6 ml) de **conjugué HRP** anti-human sL-selectin (anticorps monoclonaux anti-human sL-selectin), prêt à utilizer

10 flacons d'**étalon** human sL-selectin, lyophilisé, 50 ng/ml après reconstitution

10 flacons (50 ml) **diluant d'échantillon**

3 flacons (50 ml) de **tampon de lavage concentré** 20 x (PBS avec du Tween 20 1 %)

10 flacons (15 ml) de **solution de substrat** (tétraméthyle-benzidine)

1 flacon (100 ml) de **solution d'arrêt** (acide phosphorique 1 M)

10 **couvre-plaques** adhésifs

2. Instruction de Stockage

Conserver les réacifs du kit entre 2° et 8°C. Immédiatement après l'utilisation, les réactifs doivent être rangés au frais (2° à 8°C). La date de péremption du kit est spécifiée sur les étiquettes.
Le délai de péremption du kit ne peut être garanti que si les composants sont conservés correctement et si, en cas d'utilisation répétée d'un composant, le réactif n'a pas été contaminé lors d'une première utilisation.

3. Preventions de Sécurité pour l´Usage

- Tout réactifs doivent être considerés comme potentiellement dangereux. Pour cela il est recommandé que ce produit est utilisé que par des personnes ayant une qualification de laboratoire et qu´il soit utilisé à l'avenant au code GLP. Une tenue correspondante comme des une blouse de travail, des lunettes protectrices et des gants de travail doivent-être portés. Evitez touts contactes de réactifs avec la peau ou les yeux. En cas de contact avec les yeux ou la peau rincez immédiatement avec de l'eau. Veuillez consulter touts conseils spécifiques dans les fiches de donnés de sécurité et/ou les les règles de sécurité.
- Les réactifs sont réservés exclusivement au diagnostique et non pas au thérapeutique.
- Evitez de mélanger et d'échanger les réactifs de lots différents et de provenance différents.
- Evitez l'utilisation des réactifs perimés (voyez étiquette).
- N'exposez pas les réactifs à la lumière pendant le stockage ou l'incubation.
- Ne pas pipeter avec la bouche
- Ne pas manger, boire ou fumer dans les zones de manipulation de réactifs et d'échantillons.
- Evitez le contact de la peau et des muqueuses avec les réactifs.
- Pendant le travail avec les réactifs, utilisez des gants appropriés.
- Evitez le contact de substrats avec des métaux/oxydant.
- Evitez de gicler des liquides et la formation d'Aérosoles.
- A fin d'eviter des contaminations avec microbes ou contaminations de reactifs et d'echantillons qui pourraient rendre le test sans valeur, veuillez utiliser des pointes de pipettes jetables.
- Utilisez des tubes appropriés pour dispenser le conjugué et le substrat.
- Toute exposition aux acides inactive le conjugué.
- Pour la préparation des réactifs de l'eau déstilée ou déionisé doit être utilisée.
- La solution de substrat doit être rendue a température ambiante avant usage.
- Décontaminez et éliminez les échantillons et touts matérieaux contaminés de manière comme si ils contennaient des germes de maladies infectieuses. La méthode préferée de décontamination est par l'autoclave pour au moins une heure à 121.5 °C.
- Traitez les déchets liquides non-acidiques tel que des déchets neutralisés par l'hypochlorite de sodium (concentration finale d'hypochlorite: 1.0%). Après 30 minutes le décontamination effective est atteinte. Les déchets liquides contennant de l'acide doivent être neutralisés avant la décontamination.

4. Préparation des Réactifs

Placer **le concentré** de tampon à une température ambiente et diluer avant de commencer le test. Si des cristaux se sont formés dans le **concentré de tampon**, chauffer doucement ce dernier jusqu'à fin de les dissoluer la dissolution des cristaux totale.

4.1. Tampon de Lavage (1x)

Verser tout le contenu (50 ml) du concentré de **tampon de lavage** (20x) dans un cylindre gradué propre de 1000 ml. Porter le volume final à 1000 ml avec de l'eau distillée ou déionisée dans un alambic en verre. Mélanger doucement pour éviter la formation de mousse.

Transférer tout dans une bouteille de lavage et conserver à une température comprise entre 2º et 25ºC. Noter que le tampon de lavage reste stable pendant 30 jours.

Le tampon de lavage peut être préparé selon le tableau suivant:

Nombre de bandes	Tampon de lavage (20x) (ml)	Eau distillée (ml)
1 - 6	25	475
1 - 12	50	950

4.2. Étalon human sL-selectin

Reconstituer **étalon human sL-selectin** en ajoutant de l´eau destillée. Le volume de reconstitution est indiqué sur l'étiquette de flacon d´étalon. Agiter et mélanger avec précaution pour assurer une solubilisation homogene complete (concentration d´ étalon reconstituté = 50 ng/ml). Laisser reconstituer l´étalon pendant 10-30 min..

Apres utilization le surplus d´étalon ne doit pas être gardé et doit être eliminé.

Des **dilutions d´ étalon** peuvent être preparées directement sur la plaque de microtitration (voir 5.d) ou comme alternative dans des tubes (voir 4.2.1).

4.2.1. Dilution d´ étalon externe

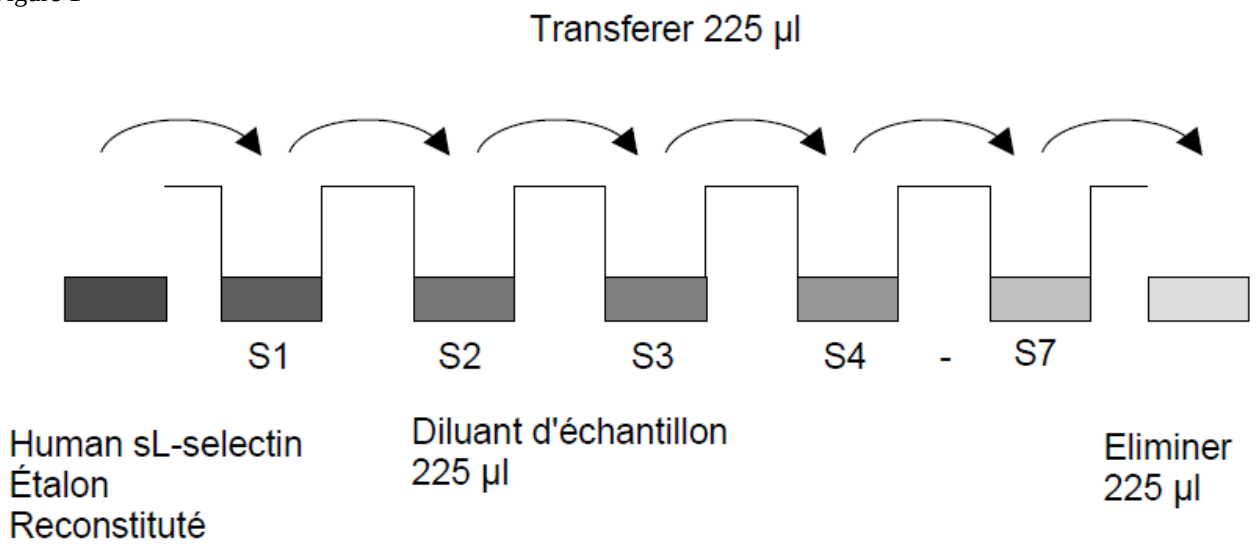
Etiquetter les tubes 7, une pour chaque point d´ étalon.

S1, S2, S3, S4, S5, S6, S7

Puis préparer séries de dilutions 1:2 pour la courbe d´ étalonnage de manière suivante: Pipeter 225 µl de Diluant d'échantillon dans chaque tube.
Pipeter 225 µl d´ étalon reconstituté (concentration d´étalon = 50 ng/ml) dans un premier tube marqué S1 et agiter (concentration d´étalon 1 = 25 ng/ml).
Pipeter 225 µl de cette dilution dans un deuxième tube marqué S2, et mélanger soigneusement avant le transfer suivant.
Répetèr des séries de dilutions 5 fois pour créer les dilutions d´étalon pour la courbe d´étalonnage (voir Figure 1).

Diluant d'échantillon sert comme contrôle vide.

Figure 1



5. Protocole de Test

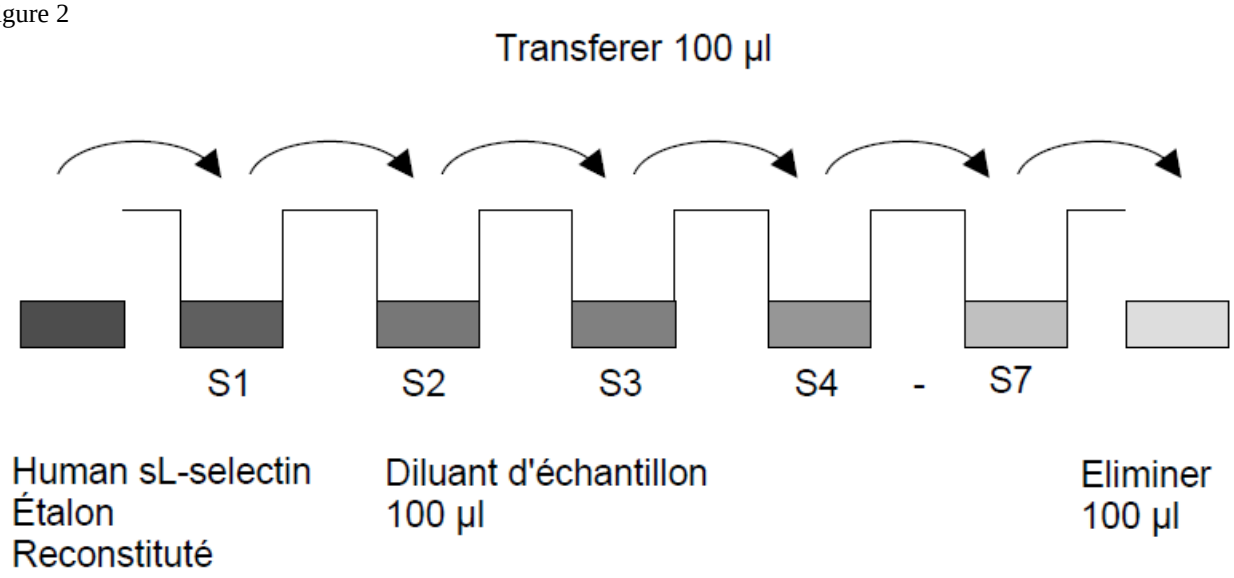
1. Prédiluer les échantillons avant que vous commençez la procedure du test. Diluer les échantillons serum plsama supernatant de la culture cellulaire à 1:100 avec du Diluant d'échantillon en utilisant le protocole de dilution suivant:

   I) 10 µl d'échantillon + 90 µl de Diluant d'échantillon

   II) 50 µl d'échantillon prédilué+ 450 µl de Diluant d'échantillon

2. Déterminer le nombre de barrettes de puits de microtitration nécessaires pour tester le nombre souhaité d'échantillons plus les barrettes nécessaires aux contrôles vides et aux étalons. Chaque échantillon, étalon et contrôle vide doit être testé en double. Retirer les barrettes de microtitration inutiles du support et les stocker à 2°-8°C dans une pochette hermétiquement refermée, avec le dessiccatif fourni.
3. Laver deux fois les barrettes de puits avec environ 400 µl de **tampon de lavage** pour chaque puits et en aspirant à fond le contenu des puits entre les lavages. Laisser le Tampon de lavage dans les puits pendant **10 - 15 secondes** avant l´aspiration. Veiller à ne pas rayer la surface des puits de microtitration.
   Après le dernier lavage, vider les barrettes de puits et les tapoter sur un tampon absorbant ou une serviette en papier pour éliminer l'excès de tampon de lavage. Utiliser les barrettes de micropuits immédiatement après le lavage ou les placer renversées sur un papier absorbant pendant 15 minutes au maximum. **Ne pas laisser sécher les puits.**
4. **<u>Dilution d´étalon sur la plaque de microtitration</u>** (Comme alternative des dilutions d´ étalon peuvent être preparées dans des tubes – 4.2.1)
   Ajouter en double 100 µl de Diluant d'échantillon dans tous les **puits d´étalon**. Pipeter en double 100 µl d´**étalon** preparé (voir Preparation d´étalon 4.2, concentration = 50.0 ng/ml) dans les puits A1 et A2 (voir Tableau 1). Mélanger bien le contenu des puits A1 et A2 par aspiration et ejection rèpetée (concentration d´étalon 1, S1 = 25.0 ng/ml), et transferer 100 µl dans les puits B1 et B2, respectivement. Transferer 100 µl dans les puits B1 et B2 (voir Figure 2) Veiller à ne pas rayer la surface des puits de microtitration. Continuer la procedure 5 fois en préparant deux séries de dilutions d´étalon human sL-selectin, de 25.0 à 0.4 ng/ml. Eliminer 100 µl du contenu des derniers puits (G1, G2).

Figure 2



   Dans le cas d´**<u>une dilution d´étalon externe</u>** (voir 4.2.1), pipeter 100 µl de cettes dilutions d´étalon (S1 – S7) dans les puits de façon montrée dans Tableau 1.

5. Ajouter 100 µl de Diluant d'échantillon dans tous les puits de contrôle vide.

Tableau 1
Exemple d´arrangement d'échantillons, d´ étalons et de contrôles vides dans les barrettes de puits de microtitration.

	1	2	3	4
A	Étalon 1 (25.0 ng/ml)	Étalon 1 (25.0 ng/ml)	Échantillon 1	Échantillon 1
B	Étalon 2 (12.5 ng/ml)	Étalon 2 (12.5 ng/ml)	Échantillon 2	Échantillon 2
C	Étalon 3 (6.3 ng/ml)	Étalon 3 (6.3 ng/ml)	Échantillon 3	Échantillon 3
D	Étalon 4 (3.2 ng/ml)	Étalon 4 (3.2 ng/ml)	Échantillon 4	Échantillon 4
E	Étalon 5 (1.6 ng/ml)	Étalon 5 (1.6 ng/ml)	Échantillon 5	Échantillon 5
F	Étalon 6 (0.8 ng/ml)	Étalon 6 (0.8 ng/ml)	Échantillon 6	Échantillon 6
G	Étalon 7 (0.4 ng/ml)	Étalon 7 (0.4 ng/ml)	Échantillon 7	Échantillon 7
H	Contrôle vide	Contrôle vide	Échantillon 8	Échantillon 8

6. Ajouter 50 µl de Diluant d'échantillon dans tous **les puits d´echantillon**.
7. Ajouter 50 µl de chaque **échantillon**, en double, dans **les puits d´echantillon**.
8. Ajouter 50 µl **Conjugué HRP** dans tous **les puits.**
9. Recouvrir avec un couvre-plaque et incuber à température ambiante (entre 18° et 25°C) pendant 2 heures, si possible sur un agitateur rotateur réglé à 100 tr/min.
10. Retirer le couvre-plaque et vider les puits. **Laver** 3 fois les barrettes de puits de microtitration comme indiqué à point c de ce protocol. Utiliser les barrettes de micropuits immédiatement après le lavage.
11. Pipeter 100 µl de **solution de substrat TMB** dans chaque puits, y compris les puits de contrôle vide.
12. Incuber les puits de microtitration à température ambiante (entre 18 et 25 C) pendant environ 10 minutes. Éviter toute exposition directe à une source de lumière intense.

    Les valeurs de densité optique au niveau de la plaque doivent être surveillées et la réaction du substrat stoppée (voir le point prochain) avant que les puits positifs ne soient plus correctement mesurables. La duree de l'incubation pour le developement de couleur doit être determiné pour chaque essai individuellement.

    Il est recommandé d'ajouter la solution stop quand une couleur bleu sombre se développe à la concentration la plus haute de la gamme étalon. Une autre alternative consiste à suivre le développement de la couleur par lecteur ELISA à 620 nm. La réaction du substrat doit être arretée dès que la DO atteint 0.9 à 0.95.
13. Arrêter la réaction enzymatique en pipetant rapidement 100 µl de **solution d'arrêt** dans chaque puits, y compris les puits de contrôle vide. Il est important que la solution d'arrêt soit répandue rapidement et uniformément dans les puits pour inactiver complètement l'enzyme. Les résultats doivent être lus immédiatement après l'ajout de la solution d'arrêt ou dans l'heure qui suit si les barrettes de microtitration sont conservées à l'obscurité entre 2 et 8 °C.
14. Lire l'absorbance de chaque puits sur un spectrophotomètre avec 450 nm comme longueur d'onde primaire (éventuellement 620 nm comme longueur d'onde de référence; 610 à 650 nm sont acceptables). Mesurer le contrôle vide du lecteur de plaque conformément aux instructions du fabricant, en utilisant les puits de contrôle vide. Déterminer l'absorbance des échantillons et des human sL-selectin.

Les échantillons ont éte diluées 1:200 en cours de test. Pour cette raison, la valeur de concéntration déterminée par la gamme étalon doit être multipliée par le facteur de dilution (x 200).

Remarque: Si la plaque n'est pas agitée pendant l'incubation, les valeurs de densité optique peuvent être inférieur aux valeurs indiqués plus haut. Néanmoins ces valeurs sont valables.

INFORMAZIONI SUL PRODOTTO E MANUALE (Italiano)

1. Reagenti Forniti

1.1. Reagenti Forniti per human sL-selectin ELISA BMS206CE (96 test)

1 busta d´alluminio con **Piastra Micropozzetti rivestita** con anticorpo monoclonale anti human sL-selectin

1 flaconcino (6 ml) di anticorpo **HRP-Coniugato,** pronto per l´uso (anticorpo monoclonale human sL-selectin)

2 flaconcini human sL-selectin **Standard** liofilizzato, 50 ng/ml previa ricostituzione diluizione

1 bottiglia (50 ml) con **Diluente del Campione**

1 bottiglia (50 ml) con **Tampone di Lavaggio concentrato** 20x (PBS con 1% Tween 20)

1 flaconcino (15 ml) di **Soluzione Substrato** (tetrametilbenzidina)

1 flaconcino (15 ml) di **Soluzione bloccante** (acido fosforico 1M)

2 **Copripiastra** adesivi

1.2. Reagenti Forniti per human sL-selectin ELISA **BMS206TENCE** (10x96 test)

10 buste d´alluminio con **Piastra Micropozzetti rivestita** con anticorpo monoclonale anti human sL-selectin

10 flaconcini (6 ml) di anticorpo **HRP-Coniugato,** pronto per l´uso (anticorpo monoclonale verso human sL-selectin)

10 flaconcini human sL-selectin **Standard** liofilizzato, 50 ng/ml previa ricostituzione

10 bottiglie (50 ml) con **Diluente del Campione**

3 bottiglie (50 ml) con **Tampone di Lavaggio concentrato** 20x (PBS con 1% Tween 20)

10 flaconcini (15 ml) di **Soluzione Substrato** (tetrametilbenzidina)

1 flaconcino (100 ml) di **Soluzione bloccante** (acido fosforico 1M)

10 **Copripiastra** adesivi

2. Istruzioni di Conservazione

Conservare i reagenti del kit a 2°-8° C. Subito dopo l'uso riporre i reagenti nel luogo di conservazione a 2°-8° C. La scadenza del kit e dei reagenti è indicata sulle etichette.
La data di scadenza dei componenti del kit può essere garantita solo se questi sono conservati correttamente e, in caso di uso ripetuto di un componente, il reagente non è stato contaminato durante la prima manipolazione.

3. Precauzioni per l'Uso

- Tutti i prodotti chimici vanno considerati come potenzialmente pericolosi. Raccomandiamo, perciò, l'utilizzo di questo prodotto solo da personale addestrato alle tecniche di laboratorio e che siano avvezze alle comuni pratiche di laboratorio. Indossare abbigliamento idoneo come camici, guanti ed occhiali. Attenzione ad evitare contatto con la pelle e gli occhi. Nel caso di contatto con pelle o occhi, immediatamente lavare con acqua. Consultare la scheda di sicurezza del prodotto per specifici consigli.
- I reagenti sono per uso in vitro diagnostico e non sono per uso terapeutico.
- Non mischiare tra loro reagenti di diversi lotti o provenienza.
- Non usare i kit dopo la data di scadenza.
- Non esporre i reagenti del kit, durante la conservazione e incubazione a forti fonti di luce.
- Non pipettare utilizzando la bocca.
- Non mangiare o fumare nell'area dove sono utilizzati i reagenti dei kit o i campioni.
- Evitare il contatto dei reagenti o campioni con la pelle o le mucose.
- Guanti di gomma o lattice dovrebbero essere sempre indossati quando si usano reagenti e campioni.
- Evitare il contatto tra il substrato del kit e agenti ossidanti e metallo.
- Evitare schizzi o produzione di aereosol.
- Per evitare contaminazione microbica o cross-contaminazione dei reagenti o dei campioni che invaliderebbero il test, usare sempre pipette e puntali mono-uso.
- Usare vaschette pulite e dedicate per la dispensare il reagente substrato.
- L'esposizione agli acidi inattiva il coniugato.
- Acqua distillata o de-ionizzata deve essere utilizzata per la preparazione dei reagenti.
- La soluzione di substrato deve essere portata a temperatura ambiente prima dell'utilizzo.
- Decontaminare ed eliminare i campioni e tutto il materiale potenzialmente contaminante perchè potrebbero contenere agenti infettanti. Il metodo preferito per la decontaminazione è l'autoclavaggio per minimo 1 ora a 121.5°C.
- Gli scarti liquidi, non contenenti acido e gli scarti neutralizzati possono essere mischiati con sodio ipoclorido in un volume finale di 1.0%. Lasciare minimo 30 minuti per l'effettiva decontaminazione. Scarti liquidi contenenti acido devono essere neutralizzati prima dell'aggiunta di sodio ipoclorido.

4. Preparazione dei Reagenti

Prima di cominciare con le procedure del test **il concentrato** dei tamponi devono essere portati a temperatura ambientale e diluiti alle concentrazioni adeguate. Se il **concentrato dei tampone** presenta cristalli in sospensione, riscaldare lievemente il tampone fino a ottenere la completa dissoluzione dei cristalli.

4.1. Tampone di Lavaggio (1x)

Versare l'intero contenuto (50 ml) del **tampone di lavaggio concentrato** (20x) in un cilindro graduato pulito da 1000 ml. Portare il volume finale a 1000 ml utilizzando acqua distillata o acqua deionizzata. Mescolare delicatamente per evitare la formazione di schiuma.

Trasferire il prodotto in una bottiglia pulita e conservare a temperature comprese fra 2°C e 25°C. Il tampone di lavaggio è stabile per 30 giorni.

Se necessario, è possibile preparare il tampone di lavaggio secondo la tabella seguente:

Numero di strip	Tampone di lavaggio (20x) (ml)	Acqua distillata (ml)
1 - 6	25	475
1 - 12	50	950

4.2. Human sL-selectin Standard

Ricostituire lo **human sL-selectin standard** aggiungendo con acqua distillata.
Il volume di ricostituzione è indicato sull'etichetta della flaconcino. Girare o mescolare gentilmente per garantire la completa ed omogenea solubilizzazione (concentrazione dello standard ricostituito = 50 ng/ml). Permettere allo standard ricostituito di riposare per 10-30 minuti. Prima di fare le diluizione mescolare bene.
Dopo l'uso, lo standard rimanente non può essere riutilizzato e deve essere buttato.

La diluizione dello standard può essere fatto direttamente nella piastra (vedi 5.d.) oppure nei tubi (vedi 4.2.1).

4.2.1. Diluizione degli Standard esterni

Etichettare 7 tubi, uno per ogni punto dello standard.

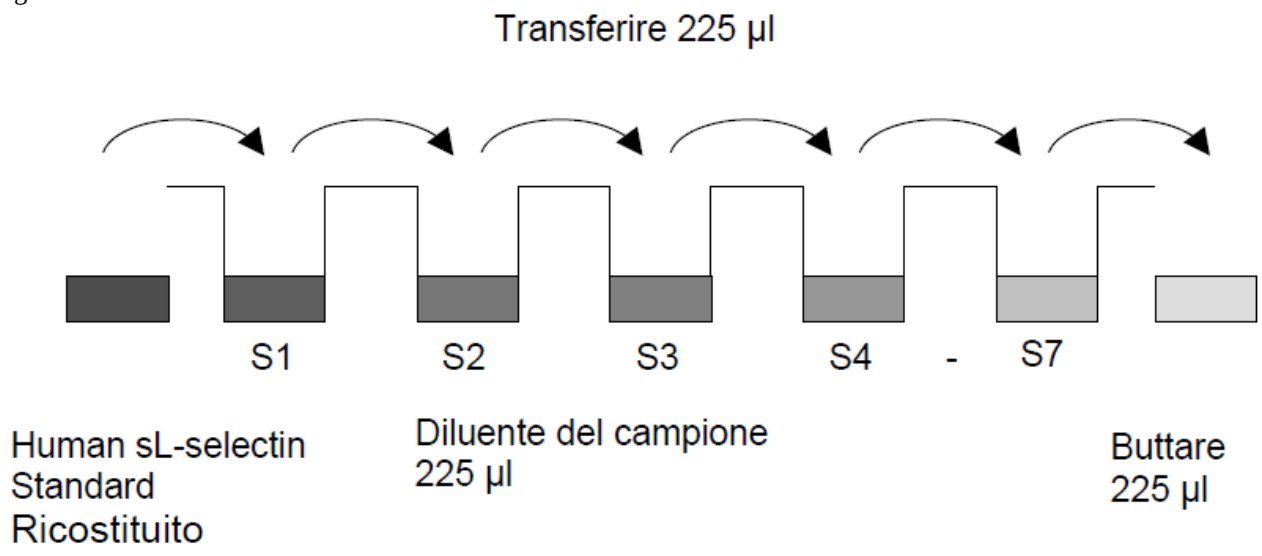
S1, S2, S3, S4, S5, S6, S7

Preparare diluizione seriali 1:2 per lo standard nel seguente modo: Pipettare 225 ul di Diluente del campione nei tutti tubi.

Pipettare 225 ul di ricostituito (concentrazione dello standard = 50 ng/ml) nel primo tubo, etichettato S1, e mescolare (concentrazione dello standard 1= 25 ng/ml).
Pipettare 225 ul di questa diluizione nel secondo tubo, etichettato S2, mischiare accuratamente prima del successivo trasferimento. Ripetere le 5 diluizioni seriali in modo da creare i punti della curva di calibrazione (vedere Figura 1)

Diluente del campione serve come bianco.

Figura 1



5. Procedura del Test

1. Diluire i campioni con Diluente del campione 1:100, secondo il seguente schema:

   I) 10 µl campione + 90 µl Diluente del campione

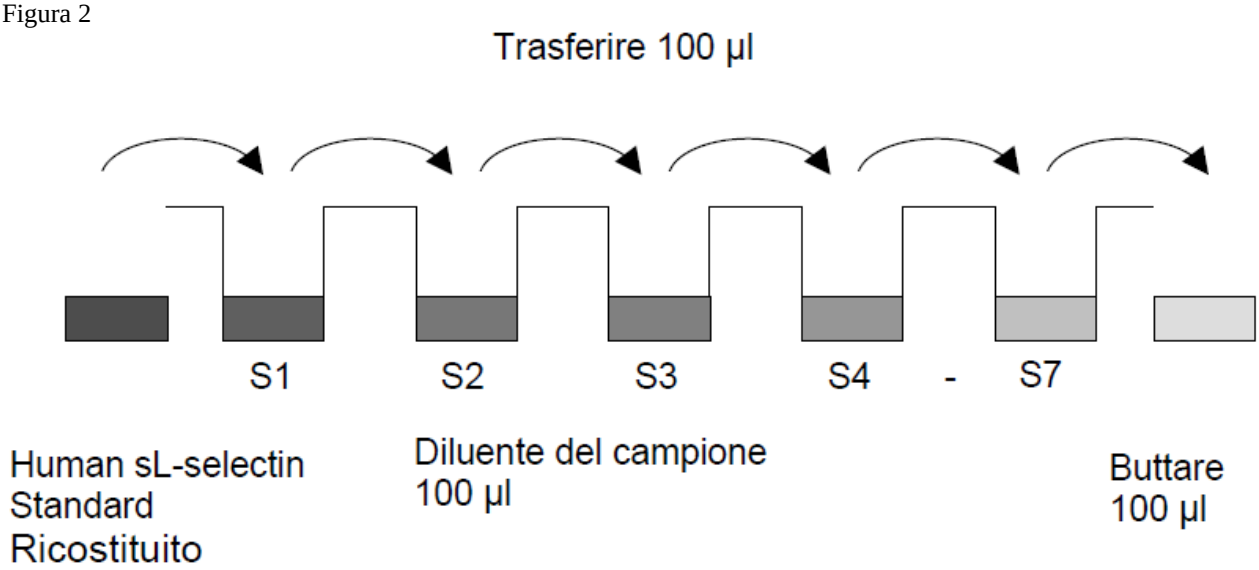
   II) 50 µl campione prediluito + 450 µl Diluente del campione

2. Stabilire il numero di strip dei micropozzetti necessarie per analizzare la quantità desiderata di campioni più le strip per i bianchi e gli standard. Tutti i campioni, gli standardi e il bianco devono essere processati in duplicato. Rimuovere dal supporto le strip micropozzetti non utilizzate e conservarle nella bustina metallica contenente la polvere essiccante, mantenendole a 2°-8°C e perfettamente sigillate.
3. Lavare due volte le strip micropozzetti utilizzando circa 400 µl di **tampone di lavaggio** per pozzetto, aspirando accuratamente il contenuto dei micropozzetti tra un lavaggio e l'altro. Permettere al tampone di lavaggio di rimanere, nei pozzetti, circa **10-15 secondi** prima dell'aspirazione. Evitare di scalfire la superficie dei micropozzetti.

   Dopo l'ultimo lavaggio, asciugare le strip micropozzetti con un tampone o carta assorbente per rimuovere il tampone di lavaggio in eccesso. Utilizzare le strip subito dopo il lavaggio o sistemarle capovolte su carta assorbente umida per non più di 15 min. **Non lasciar asciugare i pozzetti.**
4. **Diluizione dello standard in micropozetti** (alternativamente la diluizione dello standard può avvenire in tubi – vedi 4.2.1) Aggiungere 100 ul di Diluente del campione in duplicato a tutti i **pozzetti dello standard**. Pipettare 100 ul **standard** preparato (vedi preparazione dello standard 4.2.1, concentrazione = 50.0 ng/ml) in duplicato nei pozzetti A1 e A2 (vedi Tabella 1). Mescolare il contenuto dei pozzetti A1 e A2 attraverso ripetute aspirazione ed iniezioni (concentrazione dello standard 1, S1 = 25.0 ng/ml) e trasferire 100 ul, rispettivamente, ai pozzetti B1 e B2 (vedere Figura 2). Fare attenzione a non graffiare la parte interna dei pozzetti. Continuare questa procedura per 5 volte, creando due colonne di standard in diluizione con concentrazione da 25.0 a 0.4 ng/ml. Buttare 100 µl del contenuto degli ultimi pozzetti (G1 e G2).

   In caso di **diluizione esterna dello standard** (vedi 4.2.1) pipettare 100 ul di queste diluizioni standard (S1 – S7) nei pozzetti degli standard come da Tabella 1.

Figura 2



5. Dispensare 100 µl di **Diluente del campione** in duplicato ai **pozzetti de bianco**.
6. Dispensare 50 µl di Diluente del campione in duplicato ai pozzetti dei campioni.
7. Dispensare 50 µl di **campione** in duplicato ai **pozzetti dei campioni**.

Tabella 1

Tabella rappresenta un esempio dell'organizzazione dei bianchi, standardi e campioni nei pozzetti:

	1	2	3	4
A	Standard 1 (25.0 ng/ml)	Standard 1 (25.0 ng/ml)	Campione 1	Campione 1
B	Standard 2 (12.5 ng/ml)	Standard 2 (12.5 ng/ml)	Campione 2	Campione 2
C	Standard 3 (6.3 ng/ml)	Standard 3 (6.3 ng/ml)	Campione 3	Campione 3
D	Standard 4 (3.2 ng/ml)	Standard 4 (3.2 ng/ml)	Campione 4	Campione 4
E	Standard 5 (1.6 ng/ml)	Standard 5 (1.6 ng/ml)	Campione 5	Campione 5
F	Standard 6 (0.8 ng/ml)	Standard 6 (0.8 ng/ml)	Campione 6	Campione 6
G	Standard 7 (0.4 ng/ml)	Standard 7 (0.4 ng/ml)	Campione 7	Campione 7
H	Bianco	Bianco	Campione 8	Campione 8

8. Dispensare 50 µl di **HRP-coniugato** a ciascun pozzetto.
9. Coprire con un copripiastra e incubare a temperatura ambiente (18°- 25°C) per 2 ore utilizzando, se disponibile, un vortex a 400° rpm.
10. Rimuovere il copripiastra e svuotare i pozzetti. **Lavare** le strip della pozzeti 3 volte come descritto in punto 5.c. del protocollo. Procedere immediatamente al punto successivo.
11. Pipettare 100 µl di **soluzione substrato TMB** in tutti i pozzetti, inclusi quelli del blank.
12. Incubare le strip a temperatura ambiente (18°-25° C) per circa 10 minuti. Evitare l'esposizione diretta a luci intense.

    È necessario monitorare i valori O.D. a livello della piastra e interrompere la reazione del substrato (vedi il punto prossimo del protocollo) prima che i pozzetti positivi cessino di essere appropriatamente registrabili.

    La determinazione del tempo necessario per lo sviluppo del colore dev'essere fatto per ogni singolo parametro.

    Si raccomanda di aggiungere la soluzione di stop quando lo standard più elevato ha sviluppato un colore blu scuro.

    Alternativamente lo sviluppo del colore può essere monitorato con un lettore ELISA a 620 nm. La reazione del substrato deve essere bloccata non appena viene misurato un valore delle OD di 0.9 - 0.95.
13. Interrompere la reazione enzimatica pipettando rapidamente 100 µl di **soluzione bloccante** in ciascun pozzetto, inclusi i pozzetti del bianco. È importante che la soluzione bloccante si diffonda rapidamente e uniformemente attraverso i micropozzetti per inattivare completamente l'enzima. I risultati devono essere letti immediatamente dopo l'aggiunta della soluzione bloccante o entro 1 ora se le strip sono conservate in un luogo buio a 2°-8° C.
14. Leggere l'assorbanza di ciascun micropozzetto su uno spettrofotometro che utilizza 450 nm come lunghezza d'onda primaria (620 nm come lunghezza d'onda di riferimento alternativa; valori da 610 nm a 650 nm sono accettabili). Azzerare il lettore della piastra secondo le istruzioni del produttore e utilizzando i pozzetti del bianco. Determinare l'assorbanza sia dei campioni, sia degli standard di human sL-selectin.

I campioni sono stati diluiti 1: 200, quindi la concentrazione dalla curva standard risultante deve essere moltiplicata per il fattore di diluizione (x 200).

Annotazione: In caso di incubazione senza agitazione i valori di densità ottica (O.D.) potranno essere più bassi di quanto indicato sotto. Tuttavia i risultati saranno da ritenersi validi.

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