ELISA Kit
Catalog #KHS5411

Human
sICAM-1

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PURPOSE

The Invitrogen Human Soluble Intercellular Adhesion Molecule-1 (Hu sICAM-1) ELISA is an enzymed-linked immunosorbent assay for the quantitative determination of soluble Intercellular Adhesion Molecule-1 levels in cell culture supernatants, human serum, plasma, urine, amniotic fluid, bile, or other body fluids.

INTRODUCTION

Intercellular Adhesion Molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family and functions as a ligand for the Lymphocyte Function-Associated Antigen-1 (LFA-1), an alpha-beta-complex that is a member of the leukocyte integrin family of cell-cell and cell-matrix receptors. This family consists of the leukocyte adhesion glycoproteins LFA-1 which mediates lymphocyte adhesion, Mac-1 which mediates granulocyte adhesion and p150,95.

ICAM-1 is a single-chain glycoprotein with a polypeptide core of 55 kDa that can be expressed on non-hematopoietic cells of many lineages such as vascular endothelial cells, thymic epithelial cells, other epithelial cells and fibroblasts and on hematopoietic cells such as tissue macrophages, mitogen-stimulated T-lymphoblasts, germinal center B-cells and dendritic cells in tonsils, lymph nodes and Peyer’s patches. ICAM-1 is inducible on fibroblasts and endothelial cells by inflammatory mediators such as IL-1, TNF and IFN-gamma within a few hours and is correlated to the infiltration of lymphocytes into inflammatory lesions. ICAM-1 seems to be the initial marker of inflammatory reactions and is expressed prior to, and to a greater extent than is HLA-DR.

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Read entire protocol before use.

PRINCIPLE OF THE METHOD

An anti-sICAM-1 monoclonal coating antibody is adsorbed onto microwells. sICAM-1 present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated monoclonal anti-sICAM-1 antibody is added and binds to sICAM-1 captured by the first antibody. Following incubation, unbound enzyme conjugated anti-sICAM-1 is removed during a wash step and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of soluble ICAM-1 present in the sample. The reaction is terminated by addition of acid and the absorbance is measured at 450 nm. A standard curve is prepared from five sICAM-1 standard dilutions and sICAM-1 sample concentration determined.

REAGENTS PROVIDED

Reagent	*96 Test Kit*
Aluminum pouches with a microwell plate coated with monoclonal antibody (murine) to human sICAM-1.	1 pouch
HRP-Conjugated anti-sICAM-1 monoclonal (murine) antibody (60x concentrate); 0.2 mL per vial.*	1 vial
sICAM-1 Standard 10 ng/mL; 0.5 mL per vial.*	2 vials
Wash Buffer (20x concentrate) (PBS with 1% Tween 20); 50 mL per bottle.*	1 bottle
Sample Diluent (buffered protein matrix); 50 mL per bottle.*	1 bottle
Assay Buffer (20x concentrate) (PBS with 1% Tween 20 and 10% BSA); 5 mL per vial.*	1 vial
Substrate Solution (tetramethylbenzidine); 15 mL per vial.	1 vial
Stop Solution (1 M phosphoric acid); 15 mL per vial.	1 vial
Blue-Dye, Green-Dye; 0.4 mL per vial.*	2 vials
Adhesive Plate Covers.	2
* reagents contain preservative (0.01% Proclin® 300).	

STORAGE INSTRUCTIONS

Store kit reagents at 2 to 8°C. Immediately after use, remaining reagents should be returned to cold storage (2 to 8°C). Expiration date of the kit and reagents is stated on box label.

The expiration date 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.

SPECIMEN COLLECTION

Cell culture supernatants, human serum, EDTA plasma, heparinized plasma, spontaneous urine, amniotic fluid, bile, or other body fluids are suitable for use in the assay. Remove the serum or plasma from the clot or red cells, respectively, 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.

Samples must be stored frozen at -20°C to avoid loss of bioactive sICAM-1. If samples are to be run within 24 hours, they may be stored at 2 to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, frozen sera or plasma should be brought to room temperature slowly, gently mixed by hand and properly diluted in the microwells with *Sample Diluent* (1:100 see 10.b.).

To measure sICAM-1 in spontaneous urine use undiluted samples.

Cell culture media without serum component are not suitable for sICAM-1 determination with the ELISA.

For sample stability refer to 13. G (page 31).

SUPPLIES REQUIRED BUT NOT PROVIDED

1. 5 mL and 10 mL graduated pipettes.
2. 5 μL to 1000 μL adjustable single channel micropipettes with disposable tips.
3. 50 μL to 300 μL adjustable multi-channel micropipette with disposable tips.
4. Multi-channel micropipette reservoir.
5. Beakers, flasks, cylinders necessary for preparation of reagents.
6. Device for delivery of wash solution (multi-channel micropipette, wash bottle or automatic wash system).
7. Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length).
8. Glass-distilled or deionized water.
9. Statistical calculator with program to perform linear regression analysis.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techinques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory coats, 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.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.

4. Do not expose kit reagents to strong light during storage or incubation.
5. Do not pipette by mouth.
6. Do not eat or smoke in areas where kit reagents or samples are handled.
7. Avoid contact of skin and mucous membranes with kit reagents or specimens.
8. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
9. Avoid contact of substrate solution with oxidizing agents and metal.
10. Avoid splashing or generation of aerosols.
11. 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.
12. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
13. Exposure to acids will inactivate the conjugate.
14. Glass-distilled water or deionized water must be used for reagent preparation.
15. Substrate solution must be at room temperature prior to use.
16. Decontaminate and dispose of specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
17. 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.

PREPARATION OF REAGENTS

Except for the HRP-conjugate (reagent C.), the reagents should be prepared before starting the test procedure.

A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 mL) of the Wash Buffer Concentrate into a clean 1,000 mL graduated cylinder. Bring final volume to 1,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. Adjust the pH of the final solution to 7.4.

Transfer to a clean wash bottle and store at 2 to 25°C. Please note that the *Wash Buffer* is stable for 30 days. *Wash Buffer* may be prepared as needed according to the following table:

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

B. Assay Buffer

Mix the contents of the bottle well. Add contents of Assay Buffer Concentrate (5.0 mL) to 95 mL distilled or deionized water and mix gently to avoid foaming. Store at 2 to 8°C. Please note that the Assay Buffer is stable for 30 days. *Assay Buffer* may be prepared as needed according to the following table:

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

C. Preparation of HRP-Conjugate

The *HRP-Conjugate* must be diluted with *Assay Buffer* just prior to use in a clean plastic test tube.

Please note that the *HRP-Conjugate* should be used within 30 minutes after dilution. *HRP-Conjugate* may be prepared as needed according to the following table:

Number of Strips	HRP-Conjugate (mL)	Assay Buffer (mL)
1 - 6	0.05	2.95
1 - 12	0.1	5.9

D. Addition of color-producing reagents: Blue-Dye, Green-Dye

In order to help our customers to avoid any mistakes in pipetting the ELISAs, we offer a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure.

This procedure is optional. It does not in any way interfere with the test results. It is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (Blue-Dye, Green-Dye) can be added to the reagents according to the following guidelines:

(1) Diluent: Before sample dilution add the Blue-Dye at a dilution of 1:250 (see table below) to the *Sample Diluent* (1x) according to the test protocol. After addition of Blue-Dye, proceed according to the instruction booklet.

5 mL Diluent	20 µL Blue-Dye
12 mL Diluent	48 µL Blue-Dye
50 mL Diluent	200 µL Blue-Dye
60 mL Diluent	240 µL Blue-Dye

(2) HRP-Conjugate: Before dilution of the concentrated conjugate, add the Green-Dye at a dilution of 1:100 (see table below) to the *Assay Buffer* used for the final conjugate dilution. Proceed after addition of Green-Dye according to the instruction booklet, preparation of *HRP-Conjugate*.

3 mL Assay Buffer	30 µL Green-Dye
6 mL Assay Buffer	60 µL Green-Dye
12 mL Assay Buffer	120 µL Green-Dye

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the Procedural Notes/Lab Quality Control section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

1. Dilute serum or plasma samples 1:100 with *Sample Diluent* according to one of the following dilution schemes:
   I. 5 µL sample + 495 µL *Sample Diluent*
   II. 10 µL sample + 90 µL *Sample Diluent*, take 50 µL of this 1:10 diluted sample and add 450 µL *Sample Diluent*.

   Spontaneous urine samples are applied undiluted in the assay.

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* coated with monoclonal antibody (murine) to human sICAM-1 from holder and store in foil bag with the desiccant provided at 2 to 8°C sealed tightly.
3. Wash the *Microwell Strips* twice with approximately 300 µL *Wash Buffer* per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
4. After the last wash, empty wells and 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 not longer than 15 minutes. Do not allow wells to dry.
5. Add 100 µL of *Sample Diluent,* in duplicate, to all standard wells, leaving the first wells empty. Prepare standard dilutions by pipetting 200 µL of *sICAM-1 Standard,* in duplicate, into wells A1 and A2 (see Figures 1 and 2). Transfer 100 µL to wells B1 and B2, respectively. Mix the contents of wells B1 and B2 and transfer 100 µL to wells C1 and C2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure three times, creating two rows of *sICAM-1 Standard* dilutions ranging from 10 to 0.625 ng/mL. Discard 100 µL of the contents from the last microwells (E1, E2) used.

Figure 1. Preparation of *sICAM-1 Standard* dilutions:

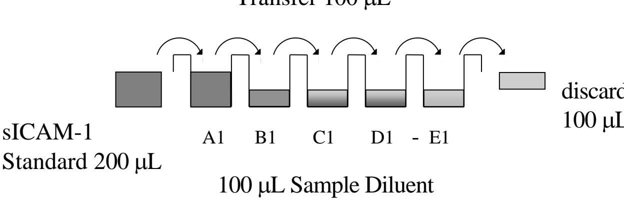


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the *Microwell Strips*:

	1	2	3	4
A	Standard 1 (10 ng/mL)	Standard 1 (10 ng/mL)	Sample 3	Sample 3
B	Standard 2 (5 ng/mL)	Standard 2 (5 ng/mL)	Sample 4	Sample 4
C	Standard 3 (2.5 ng/mL)	Standard 3 (2.5 ng/mL)	Sample 5	Sample 5
D	Standard 4 (1.25 ng/mL)	Standard 4 (1.25 ng/mL)	Sample 6	Sample 6
E	Standard 5 (0.625 ng/mL)	Standard 5 (0.625 ng/mL)	Sample 7	Sample 7
F	Blank	Blank	Sample 8	Sample 8
G	Sample 1	Sample 1	Sample 9	Sample 9
H	Sample 2	Sample 2	Sample 10	Sample 10

6. Add 100 µL of *Sample Diluent*, in duplicate, to the blank wells.
7. Add 100 µL of each 1:100 diluted sample, in duplicate, to the designated wells and mix the contents.
8. Prepare *HRP-Conjugate*. (Refer to PREPARATION OF REAGENTS 9.C.)
9. Add 50 µL of diluted *HRP-Conjugate* to all wells, including the blank wells.
10. Cover with a *Plate Cover* and incubate at room temperature (18 to 25°C) for 2 hours on a rotator set at 100 rpm, if available.
11. Remove *Plate Cover* and empty wells. Wash *Microwell Strips* 3 times according to point d. of the test protocol. Proceed immediately to the next step.
12. Pipette 100 µL of *TMB Substrate Solution* into all wells, including the blank wells.
13. Incubate the *Microwell Strips* at room temperature (18 to 25°C) for about 10 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light. **The color development on the plate should be monitored and the substrate reaction stopped (see point n. of this protocol) before positive wells are no longer properly recorded.** 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 an O.D. of 0.6 to 0.65 is reached.
14. 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 to 8°C in the dark.

15. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 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 the samples and the sICAM-1 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.

CALCULATION OF RESULTS

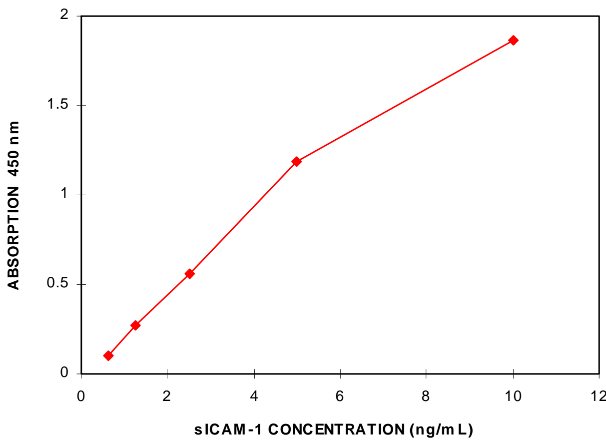
1. Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the sICAM-1 concentration on the abscissa. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating sICAM-1 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 sICAM-1 concentration.
4. For samples which have been diluted 1:100, the concentration read from the standard curve must be multiplied by the dilution factor (x 100).

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sICAM-1 levels. Such samples require further dilution of 1:200 to 1:400 with *Sample Diluent* in order to precisely quantitate the actual sICAM-1 level.

5. It is suggested that each testing facility establish a control sample of known sICAM-1 concentration and run this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.
6. A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of *Microwell Strips* assayed.

Figure 3. Representative standard curve for sICAM-1 ELISA. Recombinant sICAM-1 was diluted in serial two-fold steps in *Sample Diluent*, symbols represent the mean of three 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.



Typical data using the sICAM-1 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	sICAM-1 concentration (ng/mL)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	10	1.850	1.862	0.9
	10	1.874		
2	5	1.200	1.186	1.7
	5	1.172		
3	2.5	0.574	0.561	3.2
	2.5	0.549		
4	1.25	0.288	0.267	11.1
	1.25	0.246		
5	0.625	0.098	0.104	8.2
	0.625	0.110		
Blank	0	0.005	0.006	
	0	0.008		

LIMITATIONS OF THE PROCEDURE

1. Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
2. Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
3. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
4. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh *Wash Buffer;* fill with *Wash Buffer* as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
5. The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the *Sample Diluent*.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection of sICAM-1 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.33 ng/mL (mean of 10 independent assays).

REPRODUCIBILITY

1. Intra-assay

Reproducibility within the assay was evaluated in two independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sICAM-1. Two standard curves were run on each plate. Data below show the mean sICAM-1 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 5.6%.

Positive Sample	Experiment	sICAM-1 Concentration (ng/mL)	Coefficient of Variation (%)
1	1	162.94	6.44
	2	142.98	8.95
2	1	205.95	8.51
	2	178.08	4.84
3	1	238.82	8.09
	2	240.28	4.21
4	1	330.70	6.34
	2	328.30	4.19
5	1	665.50	11.23
	2	645.34	2.93
6	1	906.14	8.58
	2	1004.68	2.36
7	1	981.12	6.64
	2	929.24	1.93
8	1	1217.00	3.33
	2	1119.80	1.20

2. Inter-assay

Assay-to-assay reproducibility within one laboratory was evaluated in five independent experiments by two technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sICAM-1. Two standard curves were run on each plate. Data below show the mean sICAM-1 concentration and the coefficient of variation calculated on 30 determinations of each sample. The calculated overall coefficient of variation was 7.82%.

Sample	sICAM-1 Concentration (ng/mL)	Coefficient of Variation (%)
1	135.94	14.35
2	172.77	11.75
3	239.58	4.22
4	331.13	7.38
5	585.07	10.08
6	957.18	3.28
7	925.74	3.85
8	1087.58	7.65

SPIKE RECOVERY

The spike recovery was evaluated by spiking recombinant sICAM-1 into serum. As shown below, recoveries were determined in two independent experiments. The unspiked serum was used as a blank in these experiments. Average recovery ranged from 80% to 129.8% with an overall mean recovery of 103%.

Experiment 1

Sample	sICAM-1 Base Level (ng/mL)	Recovery (%) sICAM-1 Spike	
		5 ng	2.5 ng
1	1.78	129.8	121.6
2	7.81	100.0	96.6
3	12.54	80.0	93.8

Experiment 2

Sample	sICAM-1 Base Level (ng/mL)	Recovery (%) sICAM-1 Spike	
		5 ng	2.5 ng
1	0.78	112.2	107.8
2	4.50	89.7	n.d.
3	9.76	98.0	103.9

DILUTION PARALLELISM

Four serum samples with different levels of sICAM-1 were analyzed at serial two-fold dilutions (1:100 to 1:800) covering the working range of the standard curve. In the table below, the percent recovery of expected values is listed. Recoveries ranged from 83.2% to 120.9% with an overall mean recovery of 100.03%.

sICAM-1 Concentration (ng/mL)				
Sample	Dilution	Expected Value	Observed Value	% Recovery of Exp. Value
1	1:100	--	401.9	--
	1:200	201.0	203.0	101.0
	1:400	100.5	98.7	98.2
	1:800	50.3	48.2	95.8
2	1:100	--	260.9	--
	1:200	130.5	108.6	83.2
	1:400	65.2	58.1	89.1
	1:800	32.6	38.4	117.8
3	1:100	--	638.7	--
	1:200	319.4	349.7	109.5
	1:400	159.7	180.5	113.0
	1:800	79.8	96.5	120.9
4	1:100	--	177.6	--
	1:200	88.8	77.9	87.7
	1:400	44.4	37.3	84.0
	1:800	--	--	--

EXPECTED VALUES

A panel of 50 sera from healthy blood donors (male and female) was tested for sICAM-1. The detected sICAM-1 levels ranged between 129.9 and 297.4 ng/mL with a mean level of 230.3 ng/mL and a standard deviation of 47.4 ng/mL. Normal sICAM-1 levels may vary depending on the serum collective used ranging up to 400 ng/mL.

SAMPLE FREEZE-THAW STABILITY

Aliquots of serum samples (unspiked or spiked with sICAM-1) were stored at -20ºC and thawed several times, and the sICAM-1 level determined. There was no significant loss of sICAM-1 concentrations between 0 and 5 freeze-thaw cycles.

SAMPLE STORAGE STABILITY

Aliquots of a serum sample (unspiked or spiked with sICAM-1) were stored at -20°C, 2 to 8°C, room temperature and at 37°C and the sICAM-1 level determined after 24, 48 and 96 hours. There was no significant loss of sICAM-1 immunoreactivity during storage under above conditions.

COMPARISON OF SERUM AND PLASMA

From three individuals, serum as well as EDTA, citrate and heparin plasma obtained at the same time point were evaluated. sICAM-1 levels were not significantly different and therefore all these blood preparations are suitable for sICAM-1 determinations.

SPECIFICITY

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a sICAM-1 positive serum. There was no detectable cross reactivity with any of the tested proteins.

sTNF-R (60 kDa)	IFN-α2C
sTNF-R (80 kDa)	IFN-ω
IL-8/NAP-1	IL-6
TNF-α	IL-2R
TNF-β	ELAM-1
IFN-γ	L-Selectin-1

REAGENT PREPARATION SUMMARY

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

B. Assay Buffer

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

C. HRP-Conjugate

Number of Strips	HRP-Conjugate (mL)	Assay Buffer (mL)
1 - 6	0.05	2.95
1 - 12	0.1	5.90

TEST PROTOCOL SUMMARY

1. Dilute samples in *Sample Diluent* 1:100.
2. Wash *Microwell Strips* twice with *Wash Buffer*.
3. Add 100 µL *Sample Diluent*, in duplicate, to standard wells except first wells.
4. Pipette 200 µL *sICAM-1 Standard* into the first standard wells and create standard dilutions ranging from 10 to 0.625 ng/mL by transferring 100 µL from well to well. Discard 100 µL from the last wells.
5. Add 100 µL *Sample Diluent*, in duplicate, to the blank wells.
6. Add 100 µL diluted sample to designated wells.
7. Prepare *HRP-Conjugate*.
8. Add 50 µL of diluted *HRP-Conjugate* to all wells.
9. Cover *Microwell Strips* and incubate 2 hours at room temperature (18 to 25°C), shaking is recommended.
10. Prepare *TMB Substrate Solution* a few minutes prior to use.
11. Empty and wash *Microwell Strips* 3 times with *Wash Buffer*.
12. Add 100 µL of *TMB Substrate Solution* to all wells including blank wells.
13. Incubate the *Microwell Strips* for about 15 minutes at room temperature (18 to 25°C).
14. Add 100 µL *Stop Solution* to all wells including blank wells.
15. Blank microwell reader and measure color intensity at 450 nm.

Note: For samples which have been diluted 1:100, the concentration read from the standard curve must be multiplied by the dilution factor (x100). For ELISA readers that have a maximum absorbance limit of 2.0, calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sICAM-1 levels. Such samples require further dilution of 1:200 to 1:400 with Sample Diluent in order to precisely quantitate the actual sICAM-1 level.

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
Protect from Light	Protect from light		Consult accompanying documents
i	Directs the user to consult instructions for use (IFU), accompanying the product.		

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