

PRODUCT INFORMATION & MANUAL

Human sCD40L Instant ELISA

BMS239INST

Enzyme-linked immunosorbent assay for
quantitative detection of human sCD40L.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests



*Human sCD40L
Instant ELISA*

North America

Technical Support:

Research Products:

888.810.6168

858.642.2058

tech@eBioscience.com

Clinical Products:

877.726.8559

858.642.2058

tech@eBioscience.com

Customer Service:

888.999.1371

858.642.2058

info@eBioscience.com

Fax:

858.642.2046

Europe/International*

Technical Support:

+43 1 796 40 40-120

tech@eBioscience.com

Customer Service:

+43 1 796 40 40-304

europe@eBioscience.com

Fax:

+43 1 796 40 40-400



Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

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

TABLE OF CONTENTS

1	Intended Use	3
2	Summary	3
3	Principles of the Test	5
4	Reagents Provided	6
5	Storage Instructions	7
6	Specimen Collection	7
7	Materials Required But Not Provided	8
8	Precautions for Use	9
9	Preparation of Reagents and Samples	11
10	Test Protocol	12
11	Calculation of Results	16
12	Limitations	20
13	Performance Characteristics	21
14	Ordering Information	26
15	Reagent Preparation Summary	27
16	Test Protocol Summary	28

1 Intended Use

The human sCD40L Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of soluble human CD40 ligand levels in cell culture supernatants, human serum and plasma. **BMS239 is not suitable for the detection of very low levels of sCD40L in plasma; for this please refer to BMS293, human sCD40L high sensitivity ELISA.** The human sCD40L Instant ELISA is **for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

CD40 belongs to the TNF receptor superfamily. While the biological role of some of the ligand-receptor pairs in this family still remains obscure, CD40 has proven its importance.

A key role of CD40/CD40ligand interactions in immune activation, particularly in T-cell dependent B cell responses is anticipated. This molecule as well as the other ligands of the family share the property of co-stimulation of T-cell proliferation and are all expressed by activated T-cells.

The programmed cell death has been suggested to be involved in clonal elimination of self-reactive lymphocytes for the normal function of the immune system. Interaction with membrane bound self antigens may eliminate self-reactive nature B cells by apoptosis. Antigen-receptor mediated B cell apoptosis is blocked when a signal is transduced via the CD40 molecule on the B cell surface .

Because the ligand of CD40 (CD40L) is expressed on activated T helper cells, B cells may escape from apoptosis and are activated when the immune system interacts with foreign antigens, which are normally able to activate T-helper cells. Thus the CD40 - CD40L interaction plays a central role in the various phases of the B cell response to T-dependent antigens.

Taken together, B cells can participate in regulating their own destruction. Protection against Fas-dependent apoptosis afforded by immunoglobulin-receptor engagement may constitute a fail-safe

mechanism that eliminates bystander B cells activated by CD40L - expressing T cells, but ensures survival of antigen-specific B cells.

CD40 Ligand is expressed on the surface of activated CD4⁺ T cells, basophils, and mast cells. Binding of CD40L to its receptor, CD40, on the surface of B cells stimulates B-cell proliferation, adhesion and differentiation. A soluble isoform of CD40L has been shown to exist in the circulation. This soluble molecule is a homotrimer of a 18kDa protein exhibiting full activity in B cell proliferation and differentiation assays, is able to rescue B cells from apoptosis and binds soluble CD40.

CD40L is discussed in relation to a potential role in supporting B cell tumors and it has been discovered that the molecular defect in the X-linked Hyper-IgM-Syndrome is targeted to the CD40 L gene, it is functional involved in B cell hybridomas and chronic lymphocytic leukemia as well as several autoimmune diseases.

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

3 Principles of the Test

An anti-human sCD40L monoclonal coating antibody is adsorbed onto microwells. Human sCD40L present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated monoclonal anti-human sCD40L antibody binds to human sCD40L captured by the first antibody.

Following incubation unbound enzyme conjugated anti-human sCD40L is removed during a wash step and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of human sCD40L present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human sCD40L standard dilutions and human sCD40L sample concentration determined.

Figure 1

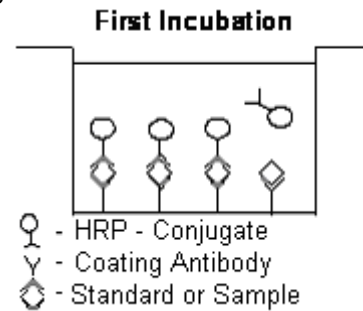


Figure 2

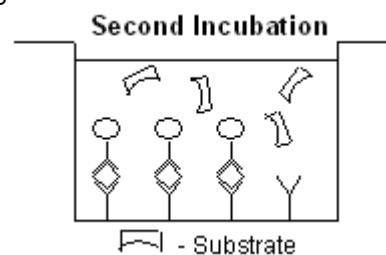
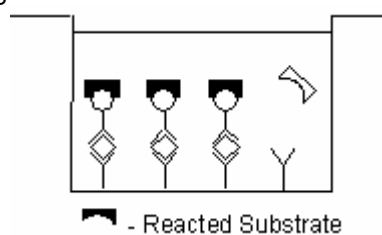


Figure 3



4 Reagents Provided

- 1 aluminium pouch with a **Microwell Plate coated with Monoclonal Antibody** (murine) to human sCD40L, **HRP-Conjugate** (anti-sCD40L monoclonal (murine) antibody) and Sample Diluent, lyophilized
- 2 aluminium pouches with a **human sCD40L Standard curve (coloured)**
- 1 bottle (25 ml) **Wash Buffer Concentrate 20x** (phosphate-buffered saline with 1% Tween 20)
- 1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (12 ml) **Sample Diluent**
(Use when an external predilution of the samples is needed)
- 1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
- 2 adhesive **Plate Covers**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatants, human serum and plasma were tested with this assay.

BMS239 is not suitable for the detection of very low levels of sCD40L in plasma; for this please refer to BMS293, human sCD40L high sensitivity ELISA.

Remove the serum or plasma from the clot or red cells as soon as possible after clotting and separation.

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

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

Samples must be stored frozen at -20°C to avoid loss of bioactive human sCD40L. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).

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

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 μ l to 1000 μ l adjustable single channel micropipettes with disposable tips
- 50 μ l to 300 μ l 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 linear regression analysis

8 Precautions for Use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

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

9.1 Wash Buffer (1x)

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

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

10 Test Protocol

- Use plate immediately after removal from -20°C!
 - Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!
 - Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results
 - Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results
 - Allow the washing buffer to sit in the wells for a few seconds before aspiration
 - Remove covers of the standard strips carefully in order that all the lyophilised pellets remain in the wells
- a. Determine the number of Microwell Strips required to test the desired number of samples plus Microwell Strips for blanks and standards (coloured). Each sample, standard, 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 -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).
 - b. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).
 - c. Add 135 µl of **distilled water** to the **sample wells**.

Table 1

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

	1	2	3	4
A	Standard 1 (10.00 ng/ml)	Standard 1 (10.00 ng/ml)	Sample 1	Sample 1
B	Standard 2 (5.00 ng/ml)	Standard 2 (5.00 ng/ml)	Sample 2	Sample 2
C	Standard 3 (2.50 ng/ml)	Standard 3 (2.50 ng/ml)	Sample 3	Sample 3
D	Standard 4 (1.25 ng/ml)	Standard 4 (1.25 ng/ml)	Sample 4	Sample 4
E	Standard 5 (0.63 ng/ml)	Standard 5 (0.63 ng/ml)	Sample 5	Sample 5
F	Standard 6 (0.32 ng/ml)	Standard 6 (0.32 ng/ml)	Sample 6	Sample 6
G	Standard 7 (0.16 ng/ml)	Standard 7 (0.16 ng/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- d. Add 15 µl of each **Sample**, in duplicate, to the designated wells and mix the contents.
- e. Cover with a **Plate Cover** and incubate at room temperature (18°C to 25°C) for 2 hours, if available on a microplate shaker at 400 rpm.
- f. Remove **Plate Cover** and empty wells. Wash the microwell strips 3 times with approximately 400 µl Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

- g. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- h. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

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

It is recommended to add the stop solution when the highest standard has developed a dark blue colour.

Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

- i. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
- j. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the

reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human sCD40L 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.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human sCD40L concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating human sCD40L 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 sCD40L concentration.
- **Samples have been diluted 1:5, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 5).**
- **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human sCD40L levels (Hook Effect). Such samples require further external predilution according to expected human sCD40L values with Sample Diluent in order to precisely quantitate the actual human sCD40L level.**
- It is suggested that each testing facility establishes a control sample of known human sCD40L concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 75 µl to the final volume per well. These 75 µl are composed of 60 µl of sample diluent plus 15 µl of the sample. This is a 1:5 dilution. The remaining 75 µl to give 150 µl are due to the addition of 75 µl conjugate to all wells.

60 µl sample diluent and 75 µl conjugate results in 135 µl reconstitution volume, addition of 15 µl sample (60 µl + 15 µl = 1:5 dilution)

Figure 4

Representative standard curve for human sCD40L Instant ELISA. Human sCD40L was diluted in serial 2-fold steps in Sample Diluent, each symbol represents the mean of 3 parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

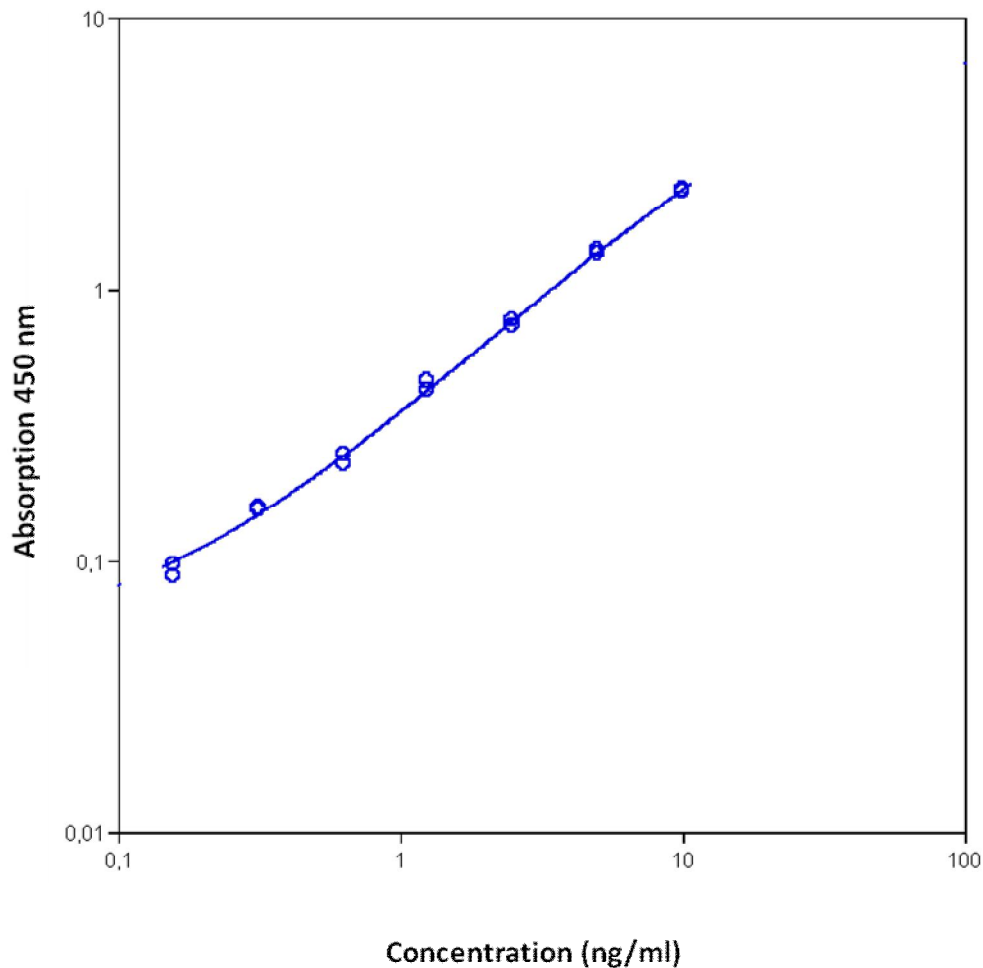


Table 2

Typical data using the human sCD40L INSTANT ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	human sCD40L Concentration (ng/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	10.00	2.301 2.336	2.319	1.1
2	5.00	1.396 1.346	1.371	2.6
3	2.50	0.728 0.766	0.747	3.6
4	1.25	0.461 0.428	0.445	5.2
5	0.63	0.245 0.228	0.237	5.1
6	0.31	0.155 0.158	0.157	1.4
7	0.16	0.089 0.097	0.093	6.1
Blank	0.00	0.023 0.029	0.026	16.3

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

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

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human sCD40L 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 0.062 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 5 serum samples containing different concentrations of human sCD40L. 2 standard curves were run on each plate. Data below show the mean human sCD40L concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.8%.

Table 3

The Mean human sCD40L concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	human sCD40L Concentration (ng/ml)	Coefficient of Variation (%)
1	1	8.8	0.8
	2	7.3	2.7
	3	7.4	5.6
2	1	14.7	13.3
	2	11.2	2.7
	3	10.6	5.6
3	1	16.5	13.4
	2	12.9	9.6
	3	13.3	11.1
4	1	19.3	1.4
	2	15.3	3.6
	3	14.6	16.2
5	1	38.7	3.4
	2	30.0	5.6
	3	32.7	7.6

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments by 3 technicians. Each assay was carried out with 6 replicates of 5 serum samples containing different concentrations of human sCD40L. 2 standard curves were run on each plate. Data below (see Table 4) show the mean human sCD40L concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall coefficient of variation was 14.2%.

Table 4

The mean human sCD40L concentration and the coefficient of variation calculated on 18 determinations of each sample.

Sample	human sCD40L Concentration (ng/ml)	Coefficient of Variation (%)
1	7.9	10.8
2	12.2	18.0
3	14.3	13.9
4	16.4	15.3
5	33.8	13.1

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human sCD40L into normal human sera. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. Average recovery ranged from 76 to 87% with an overall mean recovery of 82%.

13.4 Dilution Parallelism

4 serum samples with different levels of human sCD40L were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 85% to 102% with an overall mean recovery of 92%.

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 5 times, and the human sCD40L levels determined. There was no significant loss of human sCD40L immunoreactivity 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 sCD40L level determined after 24 h. As shown in Table 5, there was a significant loss of human sCD40L immunoreactivity during storage at RT and 37°C.

Table 5

Storage Temperature	sCD40L Serum Concentration (ng/ml)	Recovery (%)	sCD40L Spike Concentration (ng/ml)	Recovery (%)
- 20°C	5.7	100	5.8	100
2 - 8°C	4.7	82	5.6	96
RT	0.7	13	3.3	57
37°C	0.0	0	0.2	4

13.6 Comparison of Serum and Plasma

From eight individuals, serum as well as EDTA plasma, citrate plasma, and heparin plasma obtained at the same time point were evaluated. It clearly turned out that plasma preparations give results that do not correlate with the respective serum data.

13.7 Specificity

To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity observed.

13.8 Expected Values

A panel of 40 randomly selected sera from healthy blood donors (males and females) was tested for human sCD40L. The detected human sCD40L levels ranged between 0.03 and 3.98 ng/ml with a mean level of 2.13 ng/ml and a standard deviation of ± 1.0 ng/ml.

14 Ordering Information

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:

888.999.1371
858.642.2058
info@eBioscience.com

Fax:

858.642.2046

Europe/International*

Technical Support:

+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:

+43 1 796 40 40-304
europe@eBioscience.com

Fax:

+43 1 796 40 40-400



Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

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

15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

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

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 135 µl distilled water to sample wells.
- Add 15 µl Sample to designated wells.
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) on a microplate shaker at 400 rpm.
- Empty and wash microwell strips 3 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 100 µl Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

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