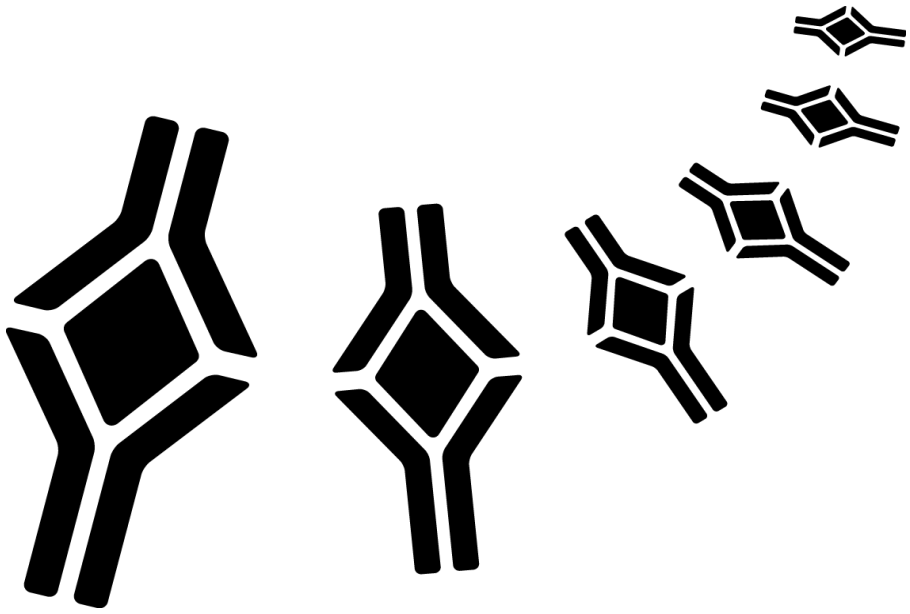
BioVendor
Research
and Diagnostic Products



HUMAN SCD40L ELISA

Product Data Sheet

Cat. No.: RAF093R

For Research Use Only

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The human sCD40L ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sCD40L. **The human sCD40L ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

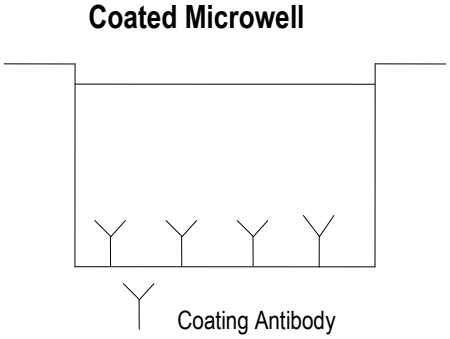
2. SUMMARY

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 CD40L gene, it is functional involved in B cell hybridomas and chronic lymphocytic leukemia as well as several autoimmune diseases.

3. PRINCIPLES OF THE TEST

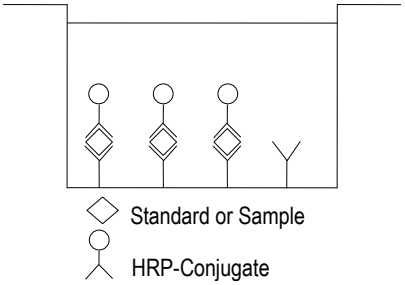
An anti-human sCD40L coating antibody is adsorbed onto microwells.

Figure 1



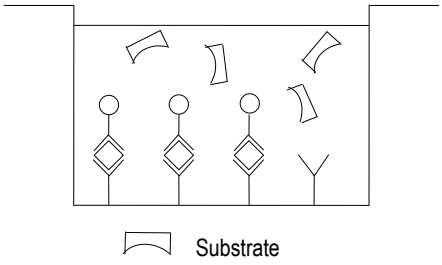
Human sCD40L present in the sample or standard binds to antibodies adsorbed to the microwells and the HRP-conjugated anti-human sCD40L antibody is added and binds to human sCD40L captured by the first antibody.

Figure 2



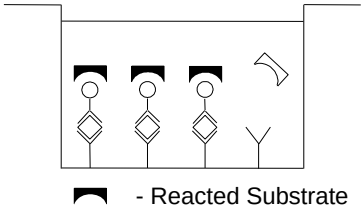
Following incubation unbound HRP-conjugated anti-human sCD40L is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 3



A coloured product is formed in proportion to the amount of human sCD40L 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 sCD40L standard dilutions and human sCD40L concentration determined.

Figure 4



4. REAGENTS PROVIDED

1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human sCD40L
1 vial (200 µl) **HRP-Conjugate** anti-human sCD40L monoclonal antibody
2 vials human sCD40L **Standard** lyophilized, 20 ng/ml upon reconstitution
1 vial **Control high,** lyophilized
1 vial **Control low,** lyophilized
1 vial (12 ml) **Sample Diluent**
1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA)
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**
2 **Dilution Plates**

5. STORAGE INSTRUCTIONS – ELISA KIT

Store kit reagents between 2° and 8°C except controls. Store lyophilized controls at -20°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C), or to -20°C, respectively. Expiry of the kit and reagents is stated on labels.
Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6. SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma (EDTA, citrate, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. *RAF093R is not suitable for the detection of very low levels of sCD40L in plasma.*
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 sCD40L. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13.5).
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)
- Microplate shaker
- 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 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 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 Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
If crystals have formed in the **Buffer Concentrates**, warm them 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. The pH of the final solution should adjust to 7.4.
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. Assay Buffer (1x)

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

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

9.3. HRP-Conjugate

Please note that the HRP-Conjugate should be used within 30 minutes after dilution.
Make a 1:100 dilution of the concentrated **HRP-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	HRP-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

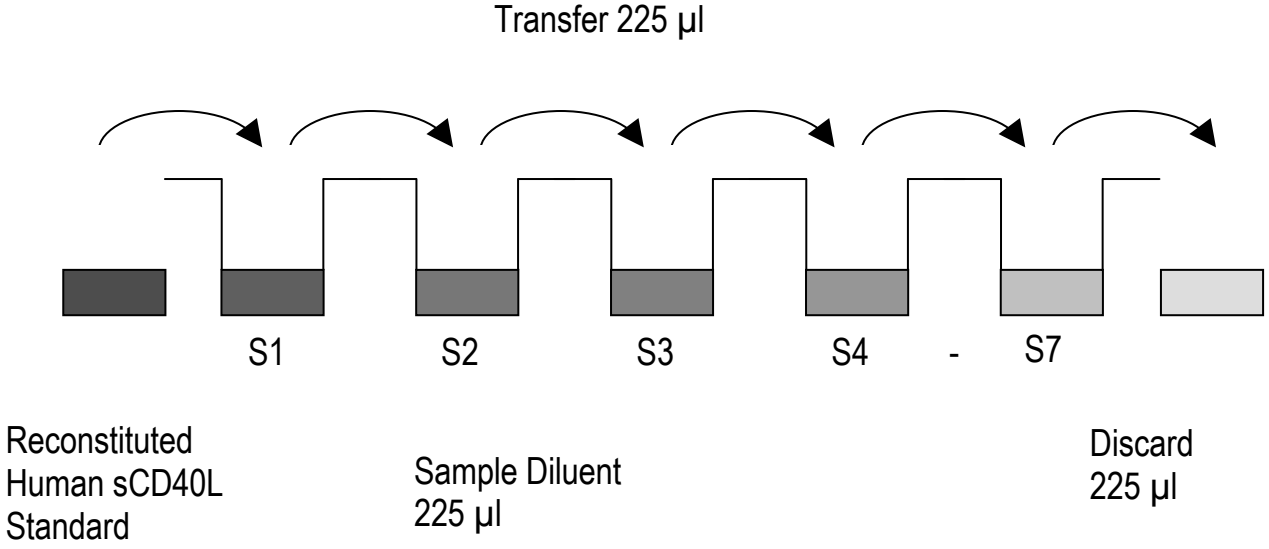
9.4. Human sCD40L Standard

Reconstitute **human sCD40L standard** by addition of distilled water.
Reconstitution volume is stated in the Quality Control Sheet. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 20 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 dilution plate (see 10.b) or alternatively in tubes (see 9.4.1).

9.4.1. 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 standard (concentration of standard = 20 ng/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 10 ng/ml). Pipette 225 µl of this dilution into the second tube, labelled 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



9.5. Controls

Reconstitute by adding 150 µl distilled water to lyophilized **controls** (10-30 minutes). Further treat the controls like your samples in the assay. For control range please refer to certificate of analysis or vial label. Store reconstituted controls aliquoted at -20°C. Avoid repeated freeze and thaw cycles.

10. TEST PROTOCOL

a. Remove **dilution plate** from the pouch. 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.

b. Prepare **standard dilutions on the dilution plate** as follows: (Alternatively the standard dilution can be prepared in tubes – see 9.4.1): Add 100 µl of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µl ofprepared **standard** (see Preparation of Standard 9.4, concentration = 20 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 = 10 ng/ml), and transfer 100 µl to wells B1 and B2, respectively (see Figure 6). Continue this procedure 5 times, creating two rows of human sCD40L standard dilutions ranging from 10.00 to 0.16 ng/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.
In case of an **external standard dilution** (see 9.4.1), pipette 100 µl of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

Figure 6

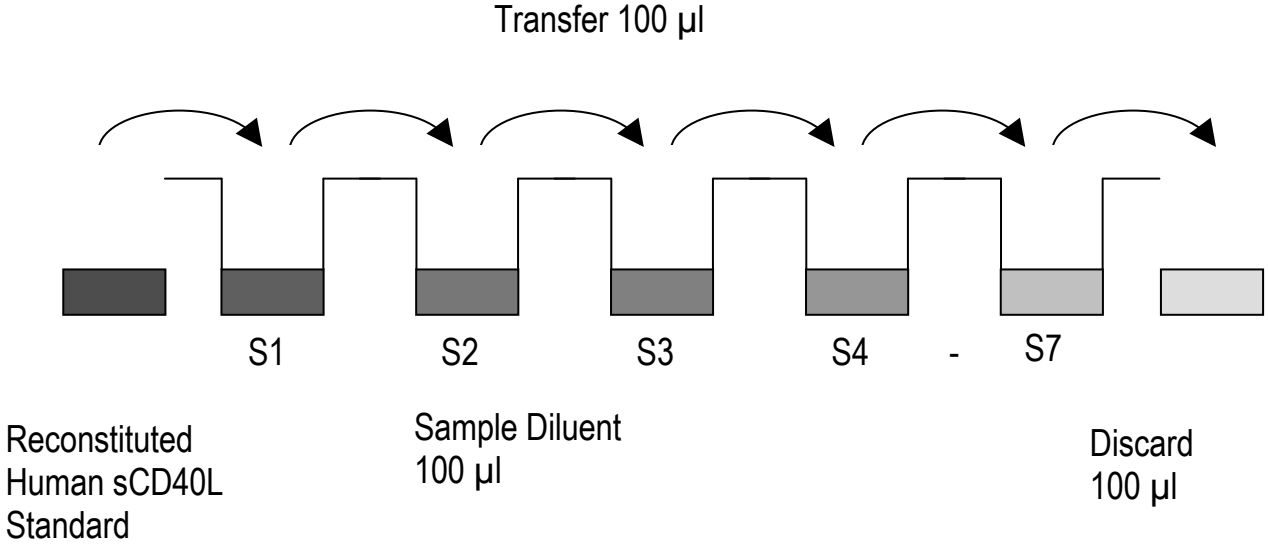


Table 1
Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips of the dilution plate:

	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 (0.31 ng/ml)	Standard 6 (0.31 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

c. Add 100 µl of **Sample Diluent** in duplicate to the **blank wells** of the dilution plate.
d. Add 80 µl of **Sample Diluent** to the **sample wells** of the dilution plate.
e. Add 20 µl of each **sample** in duplicate to the **sample wells** of the dilution plate.
f. Prepare **HRP-Conjugate** (see Preparation of HRP-Conjugate 9.3).
g. Add 100 µl of **HRP-Conjugate** to all wells of the dilution plate.
h. Remove **microwell plate coated with monoclonal antibody to human sCD40L** from the aluminium pouch. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
i. 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**.
j. **Mix** the contents of each well of the dilution plate by aspiration and ejection and **transfer** 150 µl of each reaction mixture from the dilution plate to the microwell strips coated with monoclonal antibody to human sCD40L in the same scheme as prepared on the dilution plate (see Table 1).
k. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours on a microplate shaker set at 400 rpm. If no rotator is available, the microwell plate can alternatively be incubated at 4°C over night.

l. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point i. of the test protocol. Proceed immediately to the next step.
m. Pipette 100 µl of **TMB Substrate Solution** to all wells.
n. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light. **The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.** It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.
o. 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.
p. 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 sCD40L 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 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.
- **If instructions in this protocol have been followed samples have been diluted 1:5 (20 µl sample + 80 µl Sample Diluent), 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 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 sCD40L ELISA. Human sCD40L 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.

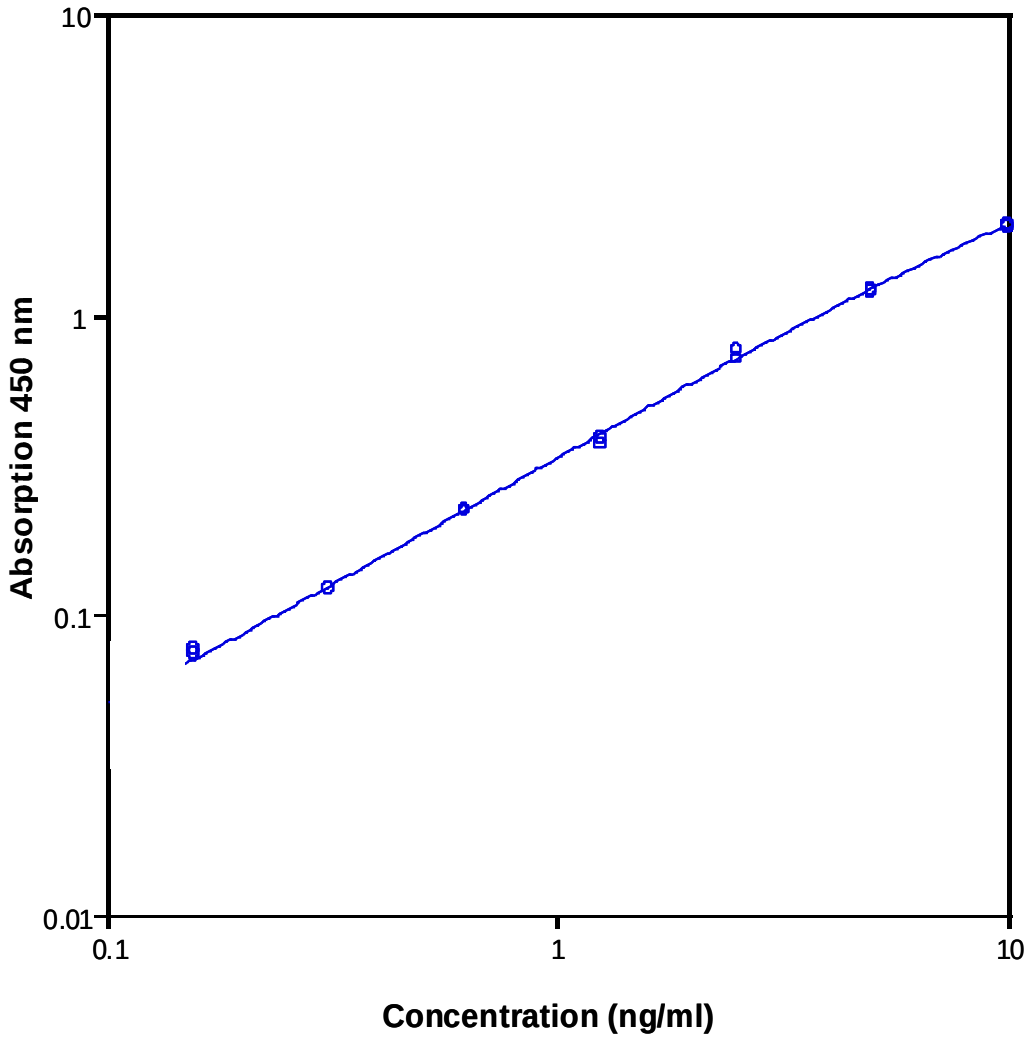


Table 2
Typical data using the human sCD40L ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human sCD40L Concentration (ng/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V.(%)
1	10.00	1.997 1.960	1.979	1.3
2	5.00	1.194 1.210	1.202	0.9
3	2.50	0.713 0.755	0.734	4.0
4	1.25	0.385 0.369	0.377	3.0
5	0.63	0.225 0.222	0.224	0.9
6	0.31	0.122 0.122	0.122	0.0
7	0.16	0.076 0.074	0.075	0.5
Blank	0	0.029 0.026	0.028	5.4

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 0.06 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 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 4.0%.

Table 3
The mean human sCD40L concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human sCD40L Concentration (ng/ml)	Coefficient of Variation (%)
1	1	14.8	1.6
	2	15.8	3.1
	3	15.0	1.4
2	1	12.6	0.5
	2	13.9	1.7
	3	13.3	5.8
3	1	10.9	1.3
	2	12.8	5.9
	3	11.8	3.9
4	1	10.2	2.2
	2	11.9	0.7
	3	11.1	3.4
5	1	7.3	0.8
	2	8.0	4.6
	3	7.4	5.1
6	1	6.6	5.9
	2	7.4	4.6
	3	7.0	8.3
7	1	4.9	5.9
	2	5.5	3.0
	3	4.7	2.6
8	1	2.9	13.6
	2	3.5	4.5
	3	2.9	4.8

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 sCD40L. 2 standard curves were run on each plate. Data below show the mean human sCD40L concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 6.8%.

Table 4
The mean human sCD40L concentration and the coefficient of variation of each sample

Sample	Mean Human sCD40L Concentration (ng/ml)	Coefficient of Variation (%)
1	15.2	3.4
2	13.3	5.0
3	11.8	8.2
4	11.1	7.7
5	7.6	5.3
6	7.0	6.0
7	5.1	8.5
8	3.1	10.1

13.3. Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human sCD40L into serum plasma. Recoveries were determined in 4 independent experiments with 6 replicates each.
The amount of endogenous human sCD40L in unspiked serum was subtracted from the spike values.
The recovery ranged from 78% to 112% with an overall mean recovery of 91%.

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 from 100% to 113% with an overall recovery of 105% (see Table 5).

Table 5

Sample	Dilution	Expected Human sCD40L Concentration (ng/ml)	Observed Human sCD40L Concentration (ng/ml)	Recovery of Expected human sCD40L Concentration (%)
1	1:5	--	14.5	--
	1:10	7.3	7.3	101
	1:20	3.6	3.7	103
	1:40	1.8	1.8	100
2	1:5	--	12.5	--
	1:10	6.3	6.7	106
	1:20	3.1	3.2	102
	1:40	1.6	1.8	112
3	1:5	--	11.1	--
	1:10	5.5	6.0	108
	1:20	2.8	2.9	106
	1:40	1.4	1.4	103
4	1:5	--	10.5	--
	1:10	5.3	5.5	104
	1:20	2.6	2.7	104
	1:40	1.3	1.5	113

13.5. Sample Stability

13.5.1. Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human sCD40L levels determined. There was no significant loss of human sCD40L 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 sCD40L level determined after 24 h. There was no significant loss of human sCD40L immunoreactivity detected during storage at -20 °C and 2-8°C.

A significant loss of human sCD40L immunoreactivity was detected during storage at RT (57% immunoreactivity) and at 37°C (4% immunoreactivity) after 24 h.

13.6. Comparison of Serum and Plasma

Serum as well as EDTA, citrate, and heparin plasma obtained from 8 individuals 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

The assay detects both natural and recombinant human sCD40L.
The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human sCD40L positive sample.
There was no cross reactivity or interference.

13.8. Expected Values

A panel of 40 sera samples from randomly selected apparently healthy 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 1.00 ng/ml.

14. REAGENT PREPARATION SUMMARY

15.1. 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

15.2. Assay Buffer (1x)

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

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

15.3. HRP-Conjugate

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

Number of Strips	HRP-Conjugate (ml)	Assay Buffer (1x) (ml)
1 – 6	0.06	5.94
1 - 12	0.12	11.88

15.4. Human sCD40L Standard

Reconstitute lyophilized **human sCD40L standard** with distilled water. (Reconstitution volume is stated in the Quality Control Sheet.)

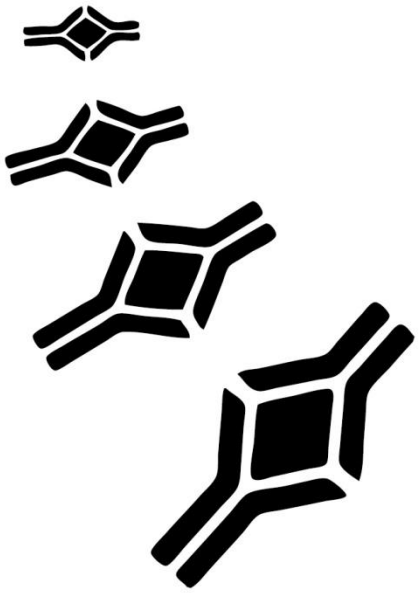
15.5. Controls

Add 150 µl distilled water to lyophilized **controls**.

15. TEST PROTOCOL SUMMARY

1. Remove dilution plate from pouch.
2. Determine the number of microwell strips required.
3. Prepare a standard dilution on the dilution plate: Add 100 µl Sample Diluent, in duplicate, to all standard wells of the dilution plate. 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 9.4.1): Pipette 100 µl of these standard dilutions in the standard wells of the dilution plate.
4. Add 100 µl Sample Diluent, in duplicate, to the blank wells of the dilution plate.
5. Add 80 µl Sample Diluent to sample wells of the dilution plate.
6. Add 20 µl sample in duplicate, to designated sample wells of the dilution plate.
7. Prepare HRP-Conjugate.
8. Add 100 µl HRP-Conjugate to all wells of the dilution plate.
9. Remove coated microwell strips from aluminium pouch.
10. Wash coated microwell strips twice with Wash Buffer.
11. Transfer 150 µl of reaction mix from dilution plate to coated microwell strips.
12. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) on a rotator set at 400 rpm.
13. Empty and wash microwell strips 3 times with Wash Buffer.
14. Add 100 µl of TMB Substrate Solution to all wells.
15. Incubate the microwell strips for about 10 minutes at room temperature (18°to 25°C).
16. Add 100 µl Stop Solution to all wells.
17. Blank microwell reader and measure colour intensity at 450 nm.

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



BioVendor – Laboratorní medicína, a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

There are BioVendor branches and distributors near you.
To find the office closest to you, visit **www.biovendor.com/contact**

BioVendor
Research
and Diagnostic Products