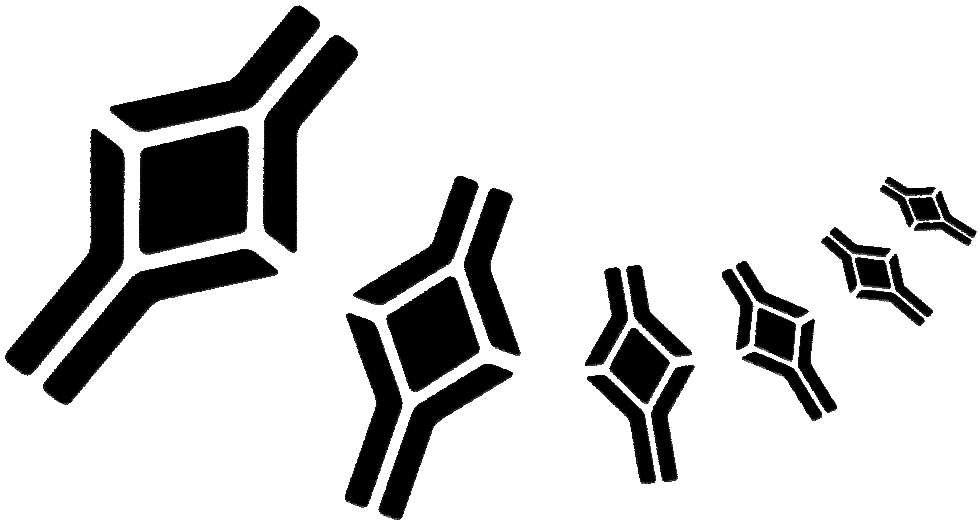
BioVendor
Research
and Diagnostic Products



Human sCD134 (OX40) ELISA

Product Data Sheet

Cat. No.: RBMS296R

For Research Use Only

VERSION 51 080708

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

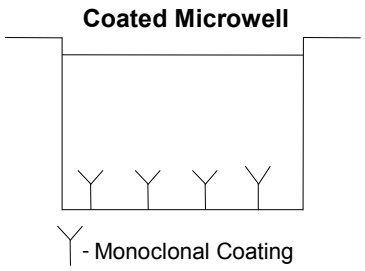
The sCD134 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human sCD134 in cell culture supernatants, human serum, plasma or other body fluids. **The sCD134 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2 SUMMARY

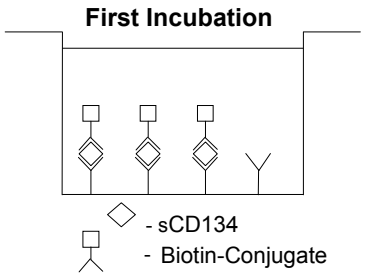
OX40 (CD134) is a member of the tumor necrosis (TNF) receptor superfamily and known to be an important costimulatory molecule expressed on activated T-cells (5, 25). Interaction of OX40 with its ligand, OX40L, is thought to be important in T cell activation through T cell/antigen-presenting cell (APC) interaction (30). Ligation of OX40 induces clonal expansion and survival of CD4 cells during primary responses, and results in the accumulation of greater numbers of memory cells with time (9).Further OX40 has been shown to be involved in the T cell adhesion to endothelium (1, 12). Induction of CD134 by Interleukin-4 has been suggested, which thus acts in a TH-2 type cytokine environment (10, 13, 20). OX40 expression is found besides T cells in a small subpopulation of macrophages, in Langerhans cells (22), and in B-cells in non Hodgkin's lymphoma (6). OX40 promotes Bcl-xL and Bcl-2 expression thus being a critical regulator of antigen-driven T cell survival (19). OX40 signaling renders adult T cell leukemia cells resistant to Fas-induced apoptosis (16). It has been described as a molecule involved in regulating immunological tolerance, which represents a major obstacle in developing effective immunotherapy against tumors (2, 3, 26). A soluble isoform of OX40 has been described (28). Measurement of this molecule may have diagnostic value in polymyositis and granulomatous myopathy (27), in T cell lymphoma (14) and lymphomatoid papulosis (8), in proliferative lupus nephritis (1), in rheumatoid arthritis (7, 30), in HIV infection (24), in viral infections of the lung (11, 21), in the regulation of graft-versus-host disease (4, 15), in myocarditis and dilated cardiomyopathy (23). The crucial role of OX40 in development of autoimmune diseases has further been shown (17, 18, 29).

3 PRINCIPLES OF THE TEST

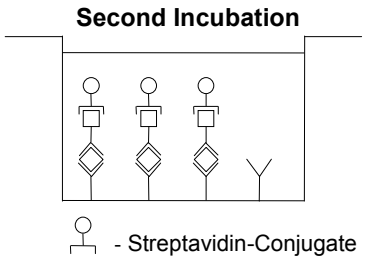
An anti-sCD134 monoclonal coating antibody is adsorbed onto microwells.



sCD134 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-sCD134 antibody is added and binds to sCD134 captured by the first antibody.



Following incubation unbound biotin conjugated anti-sCD134 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-sCD134. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.



4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with Monoclonal Antibody (murine) to human sCD134

1 vial (100 µl) **Biotin-Conjugate** anti-sCD134 monoclonal antibody

1 vial (150 µl) **Streptavidin-HRP**

2 vials **sCD134 Standard**, lyophilized, 10 ng/ml upon reconstitution

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

1 vial (5ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10 % BSA)

1 bottle (12ml) **Sample Diluent**

1 vial (7 ml) **Substrate Solution I** (tetramethyl-benzidine)

1 vial (7 ml) **Substrate Solution II** (0.02 % buffered hydrogen peroxide)

1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml each) **Blue-Dye**, **Green-Dye**, **Red-Dye**

4 adhesive **Plate Covers**

Reagent Labels

5 STORAGE INSTRUCTIONS

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.
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, plasma or other biological samples will be suitable for use in the assay. Remove serum 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.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive sCD134. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to respective chapter.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes

- 10µl to 1,000µl adjustable single channel micropipettes with disposable tips

- 50µl to 300µl 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 solutions 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 reagents.

- Exposure to acids will inactivate the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.

- Substrate solutions must be at room temperature prior to use.

- Decontaminate and dispose 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.

- 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

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. 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 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 (5.0ml) to 95ml distilled or deionized water and mix gently to avoid foaming. Store at 2º to 8ºC. 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 Biotin-Conjugate

Make a 1:100 dilution with **Assay Buffer** (reagent B) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Preparation of sCD134 Standard

Reconstitute sCD134 Standard by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard. Make sure the contents entirely dissolve by gentle swirling.

E. Preparation of Streptavidin-HRP

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution as needed according to the following table:

Number of Strips	Streptavidin- HRP (ml)	Assay Buffer (ml)
1 - 6	0.060	5.94
1 - 12	0.120	11.88

F. TMB Substrate Solution

Using clean pipettes and containers known to be metal free, dispense an equal volume of **Substrate Solution I** into **Substrate Solution II** and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue colour present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation. Substrate preparation according to assay size:

Number of Strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

G. Addition of colour-giving reagents: Blue-Dye, Green-Dye, Red-Dye

This procedure is optional, does not in any way interfere with the test results, and 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, Red-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 appropriate 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***

2. Biotin-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 Biotin-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***

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP; add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

6 ml Assay Buffer	24 µl ***Red-Dye***
12 ml Assay Buffer	48 µl ***Red-Dye***

10 TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

b. 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** to human sCD134 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. 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.

   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.

d. Add 100 µl of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (refer to preparation of reagents, 9.D.) **sCD134 Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of sCD134 standard dilutions ranging from 5000 to 78 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of sCD134 standard dilutions:

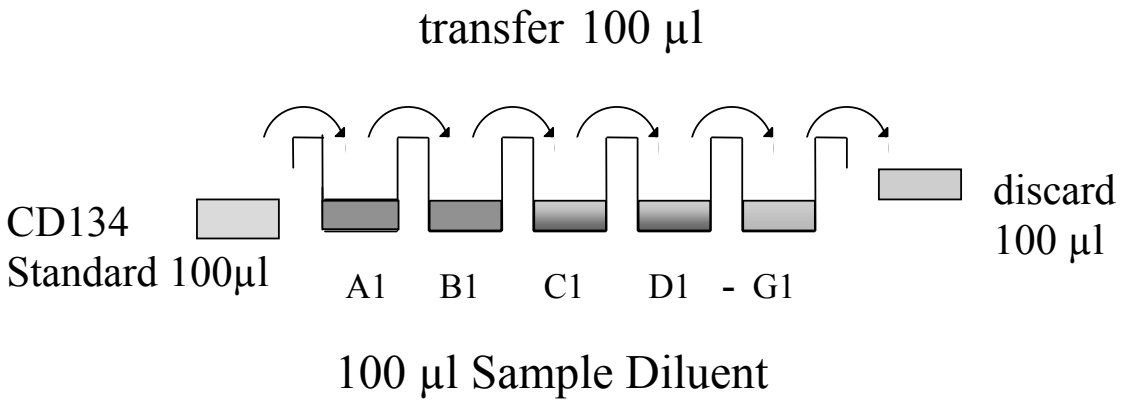


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (5000 pg/ml)	Standard 1 (5000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (2500 pg/ml)	Standard 2 (2500 pg/ml)	Sample 2	Sample 2
C	Standard 3 (1250 pg/ml)	Standard 3 (1250 pg/ml)	Sample 3	Sample 3
D	Standard 4 (625 pg/ml)	Standard 4 (625 pg/ml)	Sample 4	Sample 4
E	Standard 5 (313 pg/ml)	Standard 5 (313 pg/ml)	Sample 5	Sample 5
F	Standard 6 (156 pg/ml)	Standard 6 (156 pg/ml)	Sample 6	Sample 6
G	Standard 7 (78 pg/ml)	Standard 7 (78 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µl of **Sample Diluent** in duplicate to the blank wells.

f. Add 75µl of **Sample Diluent**, in duplicate, to the sample wells.

g. Add 25µl of each **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate** (refer to preparation of reagents).

i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature **(18° to 25°C)** for 2 hours, if available on a microplate shaker set at 100 rpm.

k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

l. Prepare **Streptavidin-HRP** (refer to preparation of reagents).

m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Cover** and incubate at room temperature **(18° to 25°C)** for 1 hour, if available on a microplate shaker set at 100 rpm.

o. Prepare **TMB Substrate Solution** a few minutes prior to use (refer to preparation of reagents).

p. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

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

r. 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 colour development on the plate should be monitored and the substrate reaction stopped (see point s. of this protocol) before positive wells are no longer properly recordable.
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 an OD of 0.6 – 0.65 is reached.

s. 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.

t. 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 sCD134 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 sCD134 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating sCD134 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 sCD134 concentration.

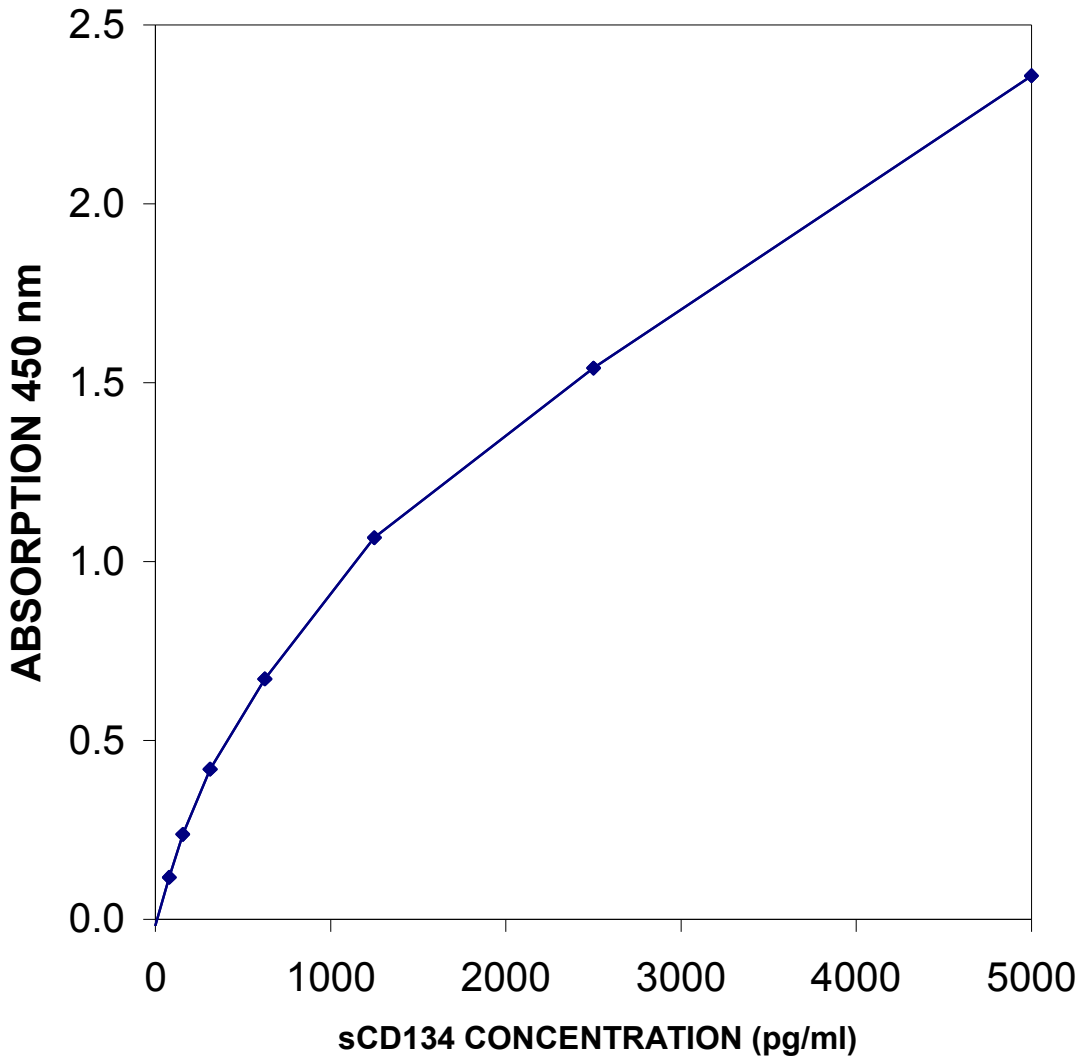
  For samples which have been diluted according to the instructions given in this manual 1:4, the concentration read from the standard curve must be multiplied by the dilution factor (x4).

  Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD134 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual sCD134 level.

  It is suggested that each testing facility establishes a control sample of known sCD134 concentration and runs 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.

- 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 sCD134 ELISA. sCD134 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 sCD134 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	sCD134 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean
1	5000	2.539	2.463
	5000	2.387	
2	2500	1.559	1.579
	2500	1.598	
3	1250	1.124	1.128
	1250	1.131	
4	625	0.814	0.767
	625	0.719	
5	313	0.511	0.474
	313	0.437	
6	156	0.292	0.292
	156	0.291	
7	78	0.179	0.173
	78	0.167	
Blank	0	0.017	0.017
	0	0.016	

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 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. 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.

13 PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of sCD134 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 less than 1.8 pg/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sCD134. Two standard curves were run on each plate. Data below show the mean sCD134 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 8.0%.

Positive Sample	Experiment	sCD134 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	9281	5
	2	10139	8
	3	9966	11
2	1	5263	9
	2	4464	7
	3	3697	4
3	1	6570	13
	2	4659	6
	3	5658	8
4	1	3837	10
	2	2801	10
	3	3068	9
5	1	2503	3
	2	2230	6
	3	2495	7
6	1	8573	7
	2	6229	8
	3	6743	10
7	1	4809	9
	2	4223	5
	3	4070	6
8	1	1845	8
	2	1585	12
	3	1782	11

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of sCD134. Two standard curves were run on each plate. Data below show the mean sCD134 concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 12.0%.

Sample	sCD134 Concentration (pg/ml)	Coefficient of Variation (%)
1	9796	4.6
2	4475	17.5
3	5629	17.0
4	3236	16.6
5	2409	6.5
6	7182	17.2
7	4367	8.9
8	1738	7.8

C. Spiking Recovery

The spiking recovery was evaluated by spiking four levels of sCD134 into 4 different pooled normal human sera. The amount of endogenous sCD134 in unspiked serum was substracted from the spike values. Mean recovery was 88%.

D. Dilution Linearity

Four serum samples with different levels of sCD134 were assayed at four serial two-fold dilutions with 4 replicates each. In the table below the per cent recovery of expected values is listed. Recoveries ranged from 87% to 125% with an overall mean recovery of 109%.

Sample	Dilution	sCD134 Concentration (pg/ml) Expected Value	Observed Value	% Recovery of Exp. Value
1	1:2	--	31176	--
	1:4	15588	16171	104
	1:8	8085	8847	109
	1:16	4423	4538	103
2	1:2	--	26701	--
	1:4	13350	14230	107
	1:8	7115	8330	117
	1:16	4165	5157	124
3	1:2	--	16152	--
	1:4	8076	8158	101
	1:8	4079	4985	122
	1:16	2492	2168	87
4	1:2	--	13339	--
	1:4	6670	8325	125
	1:8	4162	4427	106
	1:16	2213	2280	103

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored frozen at -20°C and thawed up to 5 times, and sCD134 levels determined. There was no significant loss of sCD134 by freezing and thawing up to 5 cycles of freezing and thawing.

b. Storage Stability

Aliquots of a serum (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and 37°C and the sCD134 level determined after 24 h. There was no significant loss of sCD134 immunoreactivity.

F. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a sCD134 positive serum. There was no detectable cross reactivity.

G. Expected Serum Values

There are no detectable sCD134 levels found in healthy blood donors.

14 REFERENCES

1) Aten J, Roos A, Claessen N, Schilder-Tol EF, Ten Berge IJ, Weening JJ. Strong and selective glomerular localization of CD134 ligand and TNF receptor-1 in proliferative lupus nephritis. J Am Soc Nephrol. 2000 Aug; 11 (8): 1426-38.
2) Bansal-Pakala P, Croft M. Breaking Immunological Tolerance through OX40 (CD134). Scientfic WorldJournal. 2001 Nov 6; 1 (11):
3) Bansal-Pakala P, Jember AG, Croft M. Signaling through OX40 (CD134) breaks peripheral T-cell tolerance. Nat Med. 2001 Aug; 7
4) Blazar BR, Sharpe AH, Chen AI, Panoskaltsis-Mortari A, Lees C, Akiba H, Yagita H, Killeen N, Taylor PA. Ligation of OX40 (CD134) regulates graft-versus-host disease (GVHD) and graft rejection in allogeneic bone marrow transplant recipients. Blood. 2003 May 1; 101 (9): 3741-8. Epub 2003 Jan 09.
5) Croft M. Costimulation of T cells by OX40, 4-1BB, and CD27.Cytokine Growth Factor Rev. 2003 Jun-Aug; 14 (3-4): 265-
6) Durkop H, Latza U, Himmelreich P, Stein H. Expression of the human OX40 (hOX40) antigen in normal and neoplastic tissues. Br J Haematol. 1995 Dec; 91 (4): 927-31.
7) Giacomelli R, Passacantando A, Perricone R, Parzanese I, Rascente M, Minisola G, Tonietti G. T lymphocytes in the synovial fluid of patients with active rheumatoid arthritis display CD134-OX40 surface antigen. Clin Exp Rheumatol. 2001 May-Jun; 19 (3):
8) Gniadecki R, Rossen K. Expression of T-cell activation marker CD134 (OX40) in lymphomatoid papulosis. Br J Dermatol. 2003 May; 148 (5): 885-91.
9) Gramaglia I, Jember A, Pippig SD, Weinberg AD, Killeen N, Croft M. The OX40 costimulatory receptor determines the development of CD4 memory by regulating primary clonal expansion. J Immunol. 2000 Sep 15; 165 (6): 3043-50.
10) Hoshino A, Tanaka Y, Akiba H, Asakura Y, Mita Y, Sakurai T, Takaoka A, Nakaike S, Ishii N, Sugamura K, Yagita H, Okumura K. Critical role for OX40 ligand in the development of pathogenic Th2 cells in a murine model of asthma. Eur J Immunol. 2003 Apr ; 33 (4) : 861-9.

11) Humphreys IR, Walzl G, Edwards L, Rae A, Hill S, Hussell T. A critical role for OX40 in T cell-mediated immunopathology during lung viral infection. J Exp Med. 2003 Oct 20; 198 (8): 1237-42.
12) Imura A, Hori T, Imada K, Ishikawa T, Tanaka Y, Maeda M, Imamura S, Uchiyama T. The human OX40/gp34 system directly mediates adhesion of activated T cells to vascular endothelial cells. J Exp Med. 1996 May 1; 183 (5): 2185-95.
13) Ishii N, Ndhlovu LC, Murata K, Sato T, Kamanaka M, Sugamura K. OX40 (CD134) and OX40 ligand interaction plays an adjuvant role during in vivo Th2 responses. Eur J Immunol. 2003 Sep ; 33 (9) :
14) Jones D, Fletcher CD, Pulford K, Shahsafaei A, Dorfman DM. The T-cell activation markers CD30 and OX40/CD134 are expressed in nonoverlapping subsets of peripheral T-cell lymphoma. Blood. 1999 May 15; 93 (10): 3487-93.
15) Kotani A, Ishikawa T, Matsumura Y, Ichinohe T, Ohno H, Hori T, Uchiyama T. Correlation of peripheral blood OX40+ (CD134+) T cells with chronic graft-versus-host disease in patients who underwent allogeneic hematopoietic stem cell transplantation.Blood. 2001 Nov 15; 98 (10): 3162-4.
16) Kunitomi A, Hori T, Maeda M, Uchiyama T. OX40 signaling renders adult T-cell leukaemia cells resistant to Fas-induced apoptosis.Int J Hematol. 2002 Oct; 76 (3): 260-6.
17) Murata K, Nose M, Ndhlovu LC, Sato T, Sugamura K, Ishii N. Constitutive OX40/OX40 ligand interaction induces autoimmune-like diseases. J Immunol. 2002 Oct 15; 169 (8): 4628-36.
18) Ndhlovu LC, Ishii N, Murata K, Sato T, Sugamura K. Critical involvement of OX40 ligand signals in the T cell priming events during experimental autoimmune encephalomyelitis. J Immunol. 2001 Sep 1; 167 (5): 2991-9.
19) Rogers PR, Song J, Gramaglia I, Killeen N, Croft M. OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T cells. Immunity. 2001 Sep; 15 (3): 445-55.
20) Roos A, Schilder-Tol EJ, Weening JJ, Aten J. Strong expression of CD134 (OX40), a member of the TNF receptor family, in a T helper 2-type cytokine environment. J Leukoc Biol. 1998 Oct; 64 (4): 503-
21) Salek-Ardakani S, Song J, Halteman BS, Jember AG, Akiba H, Yagi H, Croft M. OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. J Exp Med. 2003 Jul 21; 198 (2): 315-24. Epub 2003 Jul 14.

22) Sato T, Ishii N, Murata K, Kikuchi K, Nakagawa S, Ndhlovu LC, Sugamura K. Consequences of OX40-OX40 ligand interactions in langerhans cell function: enhanced contact hypersensitivity responses in OX40L-transgenic mice. Eur J Immunol. 2002 Nov; 32 (11) : 3326-35.
23) Seko Y, Ishiyama S, Nishikawa T, Kasajima T, Hiroe M, Suzuki S, Ishiwata S, Kawai S, Tanaka Y, Azuma M, Kobata T, Yagita H, Okumura K, Nagai R. Expression of tumor necrosis factor ligand superfamily costimulatory molecules CD27L, CD30L, OX40L and 4-1BBL in the heart of patients with acute moycarditis and dilated cardiomyopathy. Cardiovasc Pathol. 2002 May-Jun; 11 (3): 166-70.
24) Takahashi Y, Tanaka Y, Yamashita A, Koyanagi Y, Nakamura M, Yamamoto N. OX40 stimulation by gp34/OX40 ligand enhances productive human immunodeficiency virus type 1 infection. J Virol. 2001 Aug; 75 (15): 6748-57.
25) Takasawa N, Ishii N, Higashimura N, Murata K, Tanaka Y, Nakamura M, Sasaki T, Sugamura K. Expression of gp34 (OX40 ligand) and OX40 on human T cell clones. Jpn J Cancer Res. 2001 Apr; 92 (4): 377-82.
26) Taraban VY, Rowley TF, O'Brien L, Chan HT, Haswell LE, Green M, Tutt AL, Glennie MJ, Al-Shamkhani A. Expression and costimulatory effects of the TNF receptor superfamily members CD134 (OX40) and CD137 (4-1BB) and their role in the generation of anti-tumor immune responses. Eur J Immunol. 2002 Dec; 32 (12) : 3617-27.
27) Tateyama M, Fujihara K, Ishii N, Sugamura K, Onodera Y, Itoyama Y. Expression of OX40 in muscles of polymositis and granulomatous myopathy. J Neurol Sci. 2002 Feb 15; 194 (1): 29-34.
28) Taylor L, Schwarz H. Identification of a soluble OX40 isoform: development of a specific and quantative immunoassay. J Immunol Methods. 2001 Sep 1; 255 (1-2): 67-72.
29) Weinberg AD. OX40: targeted immunotherapy--implications for tempering autoimmunity and enhancing vaccines. Trends Immunol. 2002 Feb; 23 (2): 102-9.
30) Yoshioka T, Nakajima A, Akiba H, Ishiwata T, Asano G, Yoshino S, Yagita H, Okumura K. Contribution of OX40/OX40 ligand interaction to the pathogenesis of rheumatoid arthritis. Eur J Immunol. 2000 Oct; 30 (10): 2815-23.

15 REAGENT PREPARATION SUMMARY

A. Wash Buffer

Add **Wash Buffer Concentrate** 20 x (50 ml) 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. Biotin-Conjugate

Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Standard

Add distilled water to each vial of lyophilized **sCD134 Standard** (volume is stated on the label) as needed.

E. Streptavidin-HRP

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

F. TMB Substrate Solution

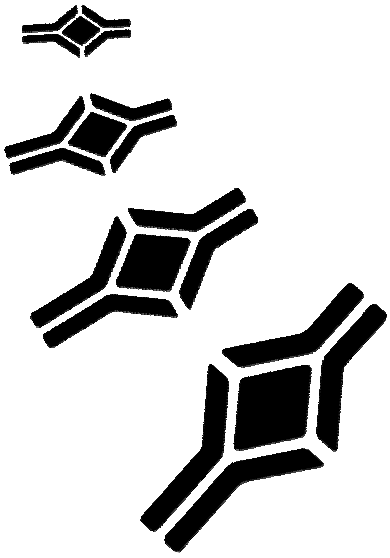
Number of Strips	Substrate Solution I (ml)	Substrate Solution II (ml)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

16 TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells
- Pipette 100µl reconstituted **sCD134 Standard** into the first wells and create standard dilutions ranging from 5000 to 78 pg/ml by transferring 100µl from well to well. Discard 100µl from the last wells
- Add 100µl **Sample Diluent**, in duplicate, to the blank wells
- Add 75µl **Sample Diluent** to the sample wells
- Add 25µl **Sample**, in duplicate, to designated wells
- Prepare **Biotin-Conjugate**
- Add 50 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature **(18° to 25°C)** on microplate shaker
- Prepare **Streptavidin-HRP**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature **(18° to 25°C)** on a microplate shaker
- Prepare **TMB Substrate Solution** few minutes prior to use
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of mixed **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for 10-20 minutes at room temperature **(18°to 25°C)** on a microplate shaker
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD134 levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual sCD134 level.

BioVendor
Research
and Diagnostic Products



HEADQUARTERS: **BioVendor Laboratorní medicína, a.s.**	**CTPark Modrice Evropska 873**	**664 42 Modrice CZECH REPUBLIC**	**Phone: +420-549-124-185 Fax: +420-549-211-460**	**E-mail:info@biovendor.com Web:www.biovendor.com**
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2405 YiYa Tower TianYu Garden, No.150	Lihe Zhong Road Guang Zhou, CHINA	Phone: +86-20-8706-3029 Fax: +86-20-8706-3016	E-mail: infoCN@biovendor.com