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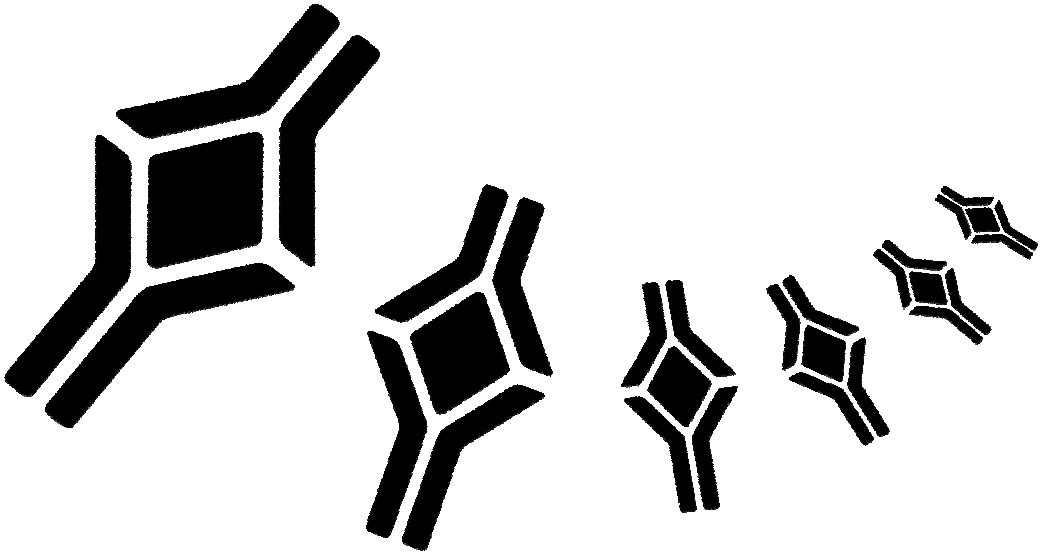


HUMAN sCD23 ELISA

Product Data Sheet

Cat. No.: RBMS227/2R

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 sCD23 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of soluble human CD23 in cell culture supernatants, human serum, plasma, or other body fluids. **The sCD23 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2 SUMMARY

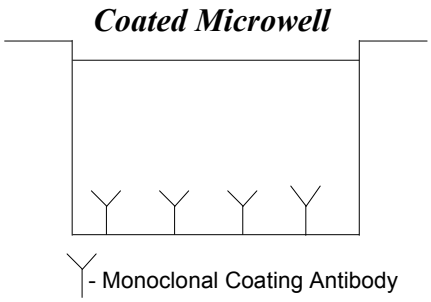
CD23 is described as a 45 kD protein found on the surface of IgM bearing B cells, eosinophils, macrophages and some T and NK cells. It is also found on EBV-transformed B cells (1). Additionally, a released form has been described (2). When first released the CD23 molecule is 35 kD; however, this form is quickly cleaved to obtain the more stable, soluble form which is 25 kD in size (3). Recently the structure of the CD23 molecule was characterized by cloning and sequencing techniques (4).

Soluble CD23 has been shown to be the B cell growth factor (BCGF). Soluble CD23 is also referred to as Blast-2 and as the low affinity IgE receptor (FCeRII) (1). It has been speculated that CD23 may up-regulate IgE synthesis in conjunction with T cell promoted interleukin-4 (1); however, the specific physiologic role of this molecule is not yet well understood.

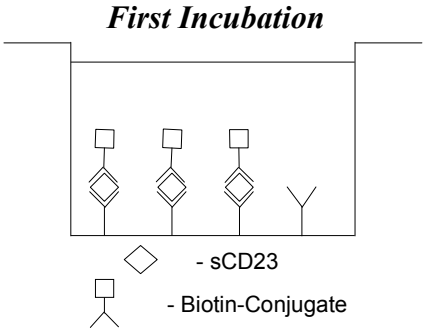
Elevated levels of CD23 have been found in research studies of samples from people with B cell-derived Chronic Lymphocytic Leukemia (B-CLL) (5, 6), with Hyper IgE Syndrome and post-Bone Marrow Transplantation samples (7, 8). CD23 levels may be proven to relate to disease course in Hairy Cell Leukemia (HCL) (9).

3 PRINCIPLES OF THE TEST

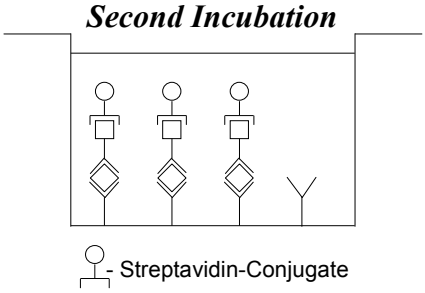
An sCD23 monoclonal coating antibody is adsorbed onto microwells.

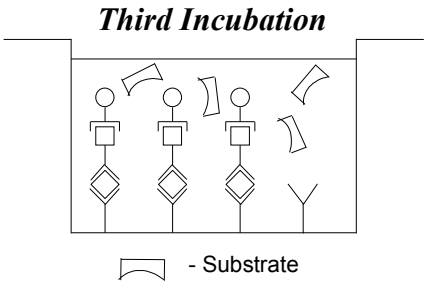


sCD23 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-sCD23 antibody is added and binds to sCD23 captured by the first antibody.

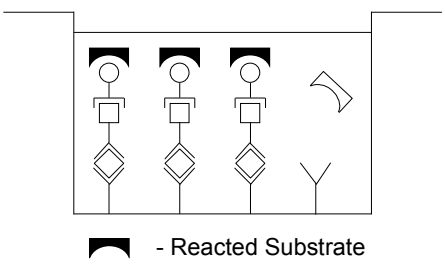


Following incubation unbound biotin conjugated anti-sCD23 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-sCD23. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.





A coloured product is formed in proportion to the amount of sCD23 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from six sCD23 standard dilutions and sCD23 sample concentration determined.



4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with Monoclonal Antibody (murine) to human sCD23

1 vial (100 µl) **Biotin-Conjugate** anti-sCD23 monoclonal (murine) antibody

2 vials **sCD23 Standard** lyophilized; 400 U/ml upon reconstitution

1 vial (150 µl) **Streptavidin-HRP**

1 vial **Control low**

1 vial **Control high**

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

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

1 vial (15 ml) **Substrate Solution**

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

3 vials (each 0.4 ml) **Blue-Dye, Green-Dye, Red-Dye**

4 adhesive **Plate Covers**

Reagent Labels

5 STORAGE INSTRUCTIONS

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). Expiry of the kit and reagents is stated on labels.

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 sCD23. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes

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

- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips

- Multichannel micropipette reservoir

- Beakers, flasks, cylinders necessary for preparation of reagents

- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)

- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

- Glass-distilled or deionized water

- Statistical calculator with program to perform 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 in vitro diagnostic use and are not for use in therapeutic procedures.

- All chemicals in this kit should be considered as potentially hazardous. We therefore recommend that this product is handled only by persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

- 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, human samples may potentially be contaminated.

- 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 reagents.

- Exposure to acids will inactivate 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 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.

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

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

B. Assay Buffer

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

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

C. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** with **Assay Buffer** (reagent B) in a clean plastic tube.
The Biotin-Conjugate may be prepared 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 Streptavidin-HRP

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

Number of Strips	Streptavidin-HRP (µl)	Assay Buffer (ml)
1 - 6	60	6
1 - 12	120	12

E. Preparation of sCD23 Standards

Reconstitute **sCD23 Standard** by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard. Swirl or mix gently to insure complete and homogeneous solubilization. Store reconstituted standard promptly at 2-8°C for up to 4 days. Aliquots held for longer periods of time should be frozen at -20°C (maximum 2 months) or at -70°C for longer storage (until expiration date).

F. Preparation of Controls

Solubilize by adding 1000 µl distilled water to each vial labeled human sCD23 **Control**. Swirl or mix gently to insure complete and homogeneous solubilization. Further dilute the controls 1:2 into the well (50µl Assay Buffer + 50µl control). 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.

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 solution 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 Assay Buffer (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

3 ml Assay Buffer	12 µl ***Blue-Dye***
6 ml Assay Buffer	24 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 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***

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** (murine) to human sCD23 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 **Assay Buffer** in duplicate to standard wells B1/2-F1/2, leaving A1/2 empty. Prepare standard dilutions by pipetting 200 µl of reconstituted (refer to preparation of reagents, 9.E.) **sCD23 Standard**, in duplicate, into wells A1 and A2 (see Figure 1 and 2). Transfer 100 µl to wells B1 and B2. Mix the contents of wells B1 and B2 by repeated aspiration and ejection, and transfer 100 µl to C1 and C2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure three times, creating two rows of sCD23 standard dilutions ranging from 400 to 12.5 U/ml. Discard 100 µl of the contents from the last microwells (F1, F2) used.

Figure 1. Preparation of sCD23 standard dilutions:

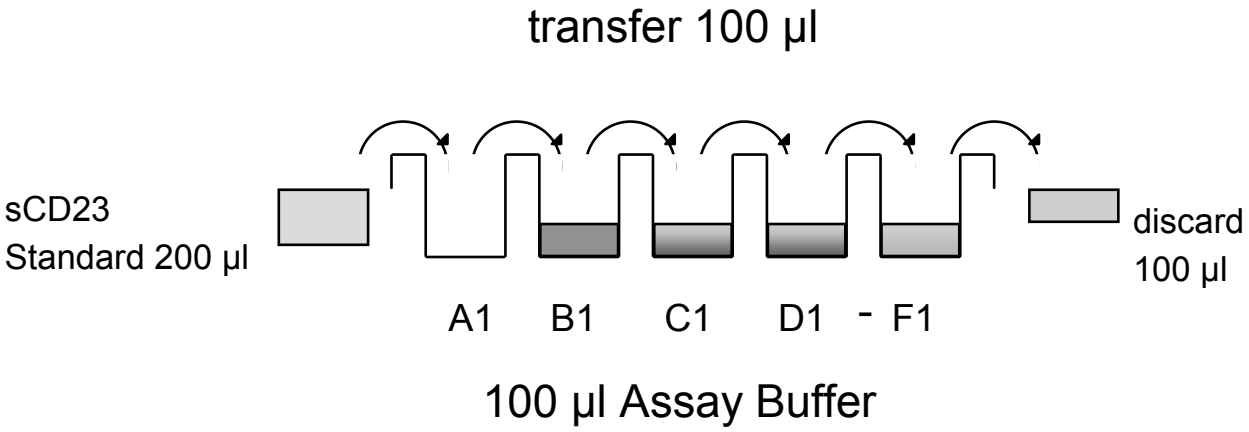


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (400 U/ml)	Standard 1 (400 U/ml)	Sample 2	Sample 2
B	Standard 2 (200 U/ml)	Standard 2 (200 U/ml)	Sample 3	Sample 3
C	Standard 3 (100 U/ml)	Standard 3 (100 U/ml)	Sample 4	Sample 4
D	Standard 4 (50 U/ml)	Standard 4 (50 U/ml)	Sample 5	Sample 5
E	Standard 5 (25 U/ml)	Standard 5 (25 U/ml)	Sample 6	Sample 6
F	Standard 6 (12.5 U/ml)	Standard 6 (12.5 U/ml)	Sample 7	Sample 7
G	Blank	Blank	Sample 8	Sample 8
H	Sample 1	Sample 1	Sample 9	Sample 9

e. Add 100 µl of **Assay Buffer** in duplicate to the blank wells.

f. Add 100 µl of each **Sample**, in duplicate, to the designated wells.

g. Prepare **Biotin-Conjugate** (refer to preparation of reagents 9.C.).

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

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

j. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to point c. of the test protocol. Proceed immediately to the next step.

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

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

m. Cover with a **Plate Cover** and incubate at room temperature (18º to 25ºC) for 1 hour, if available on a rotator set at 100 rpm.

n. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to point c. of the test protocol. Proceed immediately to the next step.

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

p. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 – 20 minutes. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point q. 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.

q. 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.

r. 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 sCD23 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 sCD23 concentration on the abscissa. Draw a best fit curve through the points of the graph.

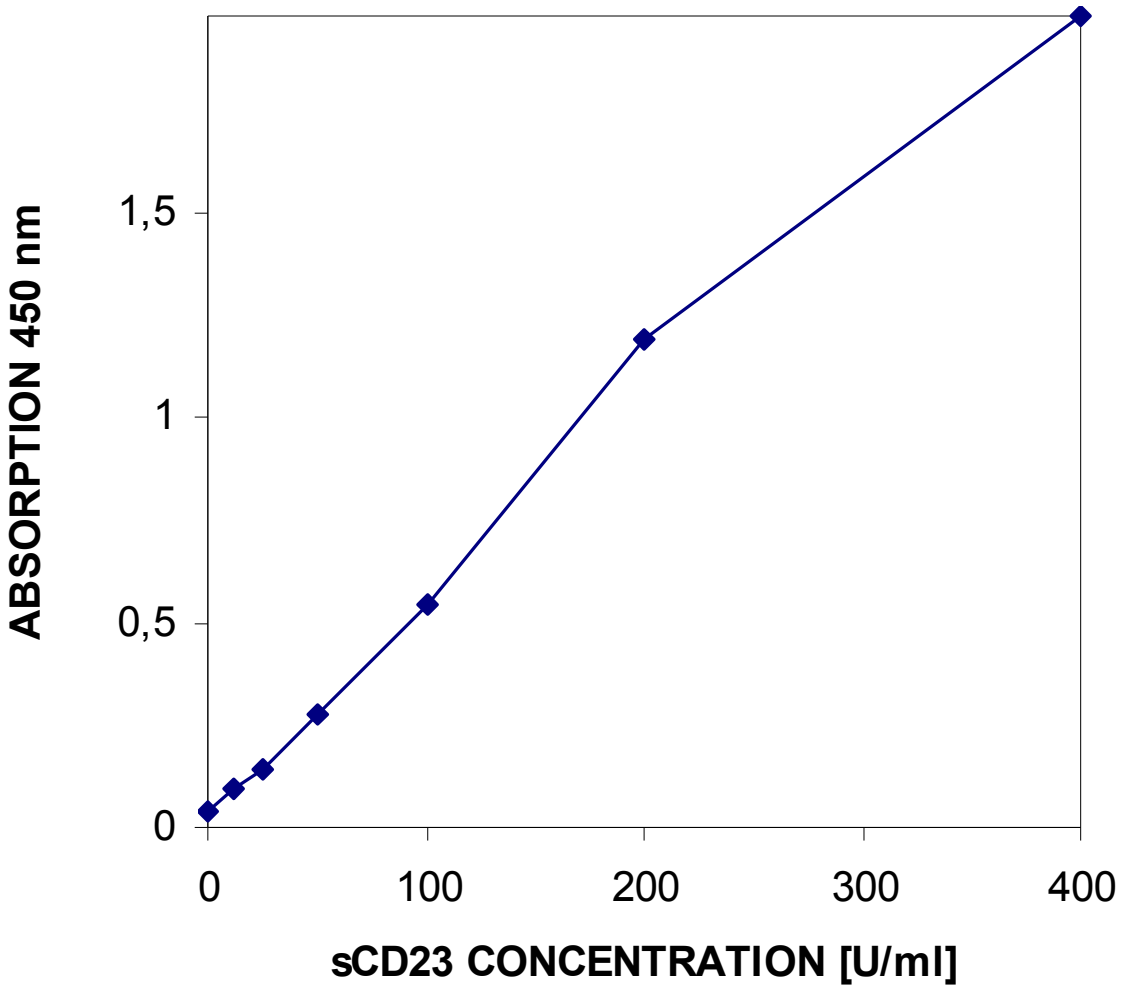
- To determine the concentration of sCD23 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding sCD23 concentration.

  Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD23 levels. Such samples require further dilution of 1:2 - 1:4 with Assay Buffer in order to precisely quantitate the actual sCD23 level.

- It is suggested that each testing facility establishes a control sample of known sCD23 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 the sCD23 ELISA. 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 sCD23 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	sCD23 Concentration (U/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	400	1.964	1.984	1.4
	400	2.003		
2	200	1.190	1.191	0.1
	200	1.191		
3	100	0.528	0.543	3.8
	100	0.557		
4	50	0.265	0.276	5.6
	50	0.287		
5	25	0.143	0.145	1.5
	25	0.146		
6	12.5	0.092	0.091	2.3
	12.5	0.089		
Blank	0	0.043	0.042	
	0	0.040		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. temperature effects). Furthermore shelf life of the kit may effect 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. 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.

- The use of immunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or mouse monoclonal antibodies of irrelevant specificity) are added to the Assay Buffer.

13 PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of sCD23 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 6.798 U/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 sCD23. Two standard curves were run on each plate. Data below show the mean sCD23 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 4.0%.

Positive Sample	Experiment	sCD23 Concentration (U/ml)	Coefficient of Variation (%)
1	1	55.78	6.3
	2	51.10	1.6
	3	50.11	4.5
2	1	71.13	4.5
	2	65.25	2.7
	3	65.38	3.1
3	1	56.88	2.1
	2	53.44	2.7
	3	52.88	4.4
4	1	67.26	4.0
	2	63.35	1.1
	3	63.78	3.9
5	1	39.93	2.0
	2	38.33	10.9
	3	39.38	10.4
6	1	67.07	3.2
	2	62.87	3.4
	3	55.35	1.6
7	1	32.10	4.5
	2	29.67	1.6
	3	27.65	10.3
8	1	34.67	4.5
	2	30.56	1.9
	3	26.56	1.7

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 sCD23. Two standard curves were run on each plate. Data below show the mean sCD23 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 6.3 %.

Sample	sCD23 Concentration (U/ml)	Coefficient of Variation (%)
1	52.33	5.8
2	67.26	5.0
3	54.40	4.0
4	64.80	3.3
5	39.21	2.1
6	61.76	9.6
7	29.81	7.5
8	30.60	13.3

C. Spiking Recovery

The spiking recovery was evaluated by spiking four levels of sCD23 into four normal human sera. Recoveries were determined in three independent experiments with 4 replicates each. The amount of endogenous sCD23 in unspiked serum was substracted from the spike values. Recoveries ranged from 81.4 to 119.9% with an overall mean recovery of 92.5%.

D. Dilution Linearity

Four serum samples with different levels of sCD23 were assayed at three serial two-fold dilutions with 4 replicates each. In the table below the per cent recovery of expected values is listed. Recoveries ranged from 88.3% to 113.0% with an overall mean recovery of 99.7 %.

Sample	Dilution	sCD23 Concentration (U/ml) Expected Value	Observed Value	% Recovery of Exp. Value
1	-	-	87.3	-
	1:2	43.6	40.4	92.5
	1:4	21.8	19.3	88.3
2	-	-	43.7	-
	1:2	21.9	20.2	92.6
	1:4	10.9	12.1	110.5
3	-	-	131.4	-
	1:2	65.7	66.3	101.0
	1:4	32.8	32.4	98.8
4	-	-	92.0	-
	1:2	46.0	46.4	101.0
	1:4	23.0	26.0	113.0

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed up to 5 times, and sCD23 levels determined. There was no significant loss of sCD23 by freezing and thawing up to 5 times.

b. Storage Stability

Aliquots of a serum sample (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the sCD23 level determined after 24 h. There was no significant loss of sCD23 immunoreactivity during storage under above conditions.

F. Comparison of Serum and Plasma

From eight individuals, serum as well as EDTA, citrate and heparin plasma obtained at the same time point were evaluated. All these blood preparations were found suitable for sCD23 determinations, although sCD23 values in citrate and EDTA plasmas were slightly lower than serum values. It is, therefore, highly recommended to assure the uniformity of blood preparations.

G. Specificity

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

H. Expected Values

A panel of 8 sera from apparently healthy blood donors (males and females) was tested for sCD23. The serum levels ranged from below 10 U/ml to 91 U/ml with an average value of 47 U/ml. The normal levels measured may however vary with the sample collective used.

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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 the volume of distilled water as stated on label to each vial of lyophilized **sCD23 Standard** as needed.

E. Streptavidin-HRP

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

16 TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Assay Buffer**, in duplicate, to standard wells B1/2-F1/2, leaving A1/2 empty
- Pipette 200 µl reconstituted **sCD23 Standard** into the wells A1 and A2 and create standard dilutions ranging from 400 to 12.5 units/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl **Assay Buffer**, in duplicate, to the blank wells
- Add 100 µl **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 4 times with **Wash Buffer**
- Add 100 µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C) on microplate shaker
- Empty and wash microwell strips 4 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for 10-20 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: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD23 levels. Such samples require further dilution of 1:2-1:4 with Assay Buffer in order to precisely quantitate the actual sCD23 level.

NOTES

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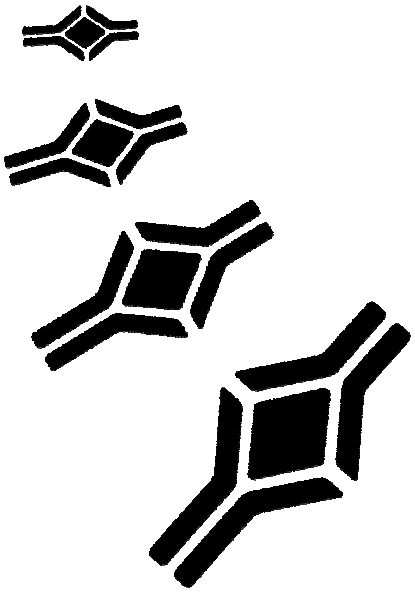

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