

---

*Please use only the valid version of the package insert provided with the kit.*

---

#### Intended Use

The human sCD23 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sCD23. **The human sCD23 ELISA is for in vitro use. Not for use in therapeutic procedures. In the United States, this kit is intended for Research Use Only.**

#### Summary

CD23 is described as a 45 kDa 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 kDa; however, this form is quickly cleaved to obtain the more stable, soluble form which is 25 kDa 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 (FCεRII) (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).

#### Principles of the Test

An anti-human sCD23 coating antibody is adsorbed onto microwells.

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

Following incubation unbound biotin-conjugated anti-human sCD23 antibody is removed during a wash step.

Streptavidin-HRP is added and binds to the biotin-conjugated anti-human sCD23 antibody.

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 human sCD23 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 6 human sCD23 standard dilutions and human sCD23 sample concentration determined.

#### Reagents Provided

##### Reagents for human sCD23 ELISA (96 tests)

- 1 aluminium pouch with a **Microwell Plate** coated with monoclonal antibody to human sCD23
- 1 vial (100 µl) **Biotin-Conjugate** anti-human sCD23 monoclonal antibody
- 1 vial (150 µl) **Streptavidin-HRP**
- 2 vials human sCD23 **Standard** lyophilized, 400 U/ml upon reconstitution
- 1 vial **Control high**, lyophilized
- 1 vial **Control low**, lyophilized

- 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 (12 ml) **Stop Solution** (1M Phosphoric acid)
- 1 vial (0.4 ml) **Blue-Dye**
- 1 vial (0.4 ml) **Green-Dye**
- 1 vial (0.4 ml) **Red-Dye**
- 4 **Adhesive Films**

#### 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), controls 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.

#### 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.

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 0).

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 sCD23. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 0).

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

#### 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)
- 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

---

#### 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 statement(s) for specific advice.
- Reagents are intended for in vitro use and are not for use in 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.

#### 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.

#### 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:

Revised 26 Aug. 2009 (Vers. 2.0)

---

---

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

---

**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

**Biotin-Conjugate**

**Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.**

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

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

**Streptavidin-HRP**

**Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.**

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

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

**Human sCD23 Standard**

Reconstitute **human sCD23 standard** by addition of distilled water.

Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilisation (concentration of reconstituted standard = 400 U/ml).

After usage remaining standard cannot be stored and has to be discarded.

**Standard dilutions** can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 0).

**External Standard Dilution**

Label 5 tubes, one for each standard point.

S2, S3, S4, S5, S6

Then prepare 1:2 serial dilutions for the standard curve as follows:

Pipette 225 µl of Assay Buffer (1x) into tubes S2 – S6.

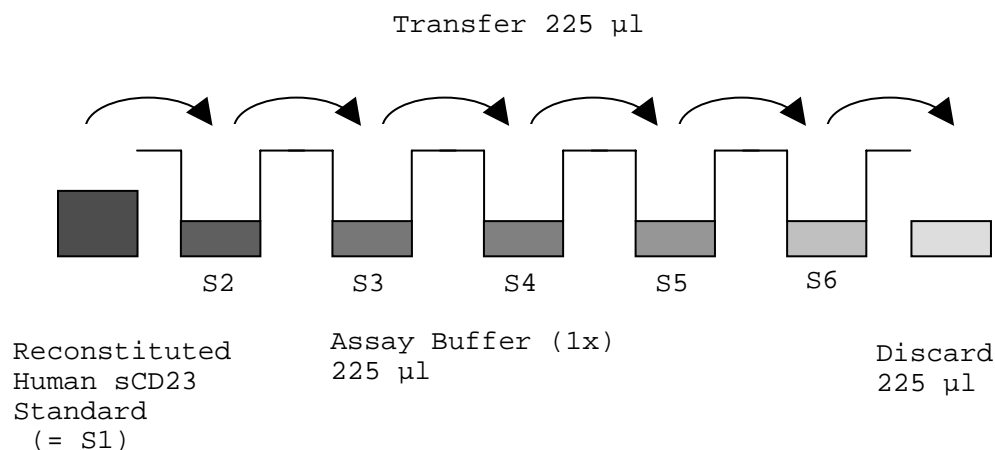
Pipette 225 µl of reconstituted standard (serves as the highest standard S1, concentration of standard 1 = 400 U/ml) into the first tube, labelled S2, and mix (concentration of standard 2 = 200 U/ml).

Pipette 225 µl of this dilution into the second tube, labelled S3, and mix thoroughly before the next transfer.

Repeat serial dilutions 3 more times thus creating the points of the standard curve (see Figure 1).

Assay Buffer (1x) serves as blank.

Figure 1



## Controls

Reconstitute by adding 1000 µl distilled water to lyophilized controls. Swirl or mix gently to ensure complete and homogeneous solubilization.

Predilute the controls 1:2 with Assay Buffer (1x) according to the following dilution scheme:

150 µl reconstituted control + 150 µl Assay Buffer (1x)

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.

## Addition of Colour-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

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

**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 standard and 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 Assay Buffer (1x)	20 µl <i>Blue-Dye</i>
12 ml Assay Buffer (1x)	48 µl <i>Blue-Dye</i>
50 ml Assay Buffer (1x)	200 µl <i>Blue-Dye</i>

**2. Biotin-Conjugate:** Before dilution of the concentrated Biotin-Conjugate, add the *Green-Dye* at a dilution of 1:100 (see table below) to the Assay Buffer (1x) 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 (1x)	30 µl <i>Green-Dye</i>
6 ml Assay Buffer (1x)	60 µl <i>Green-Dye</i>

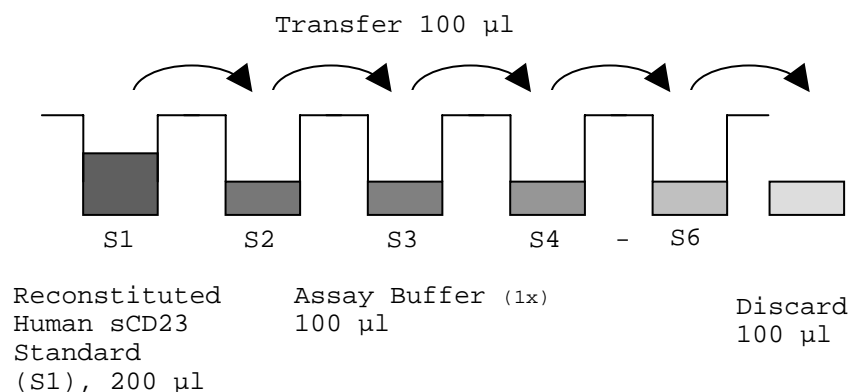
**3. Streptavidin-HRP:** Before dilution of the concentrated Streptavidin-HRP, add the *Red-Dye* at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of *Red-Dye* according to the instruction booklet: Preparation of Streptavidin-HRP.

6 ml Assay Buffer (1x)	24 µl <i>Red-Dye</i>
12 ml Assay Buffer (1x)	48 µl <i>Red-Dye</i>

# Test Protocol

- a. 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 from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
- b. 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.**
- c. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 0): Add 100 µl of Assay Buffer (1x) in duplicate to **standard wells** B1/2-F1/2, leaving A1/A2 empty. Pipette 200 µl of prepared **standard** (see Preparation of Standard 0, concentration = 400 U/ml) in duplicate into well A1 and A2 (see Table 1). 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 wells C1 and C2, respectively (see Figure 2). Take care not to scratch the inner surface of the microwells. Continue this procedure 3 times, creating two rows of human sCD23 standard dilutions ranging from 400.0 to 12.5 U/ml. Discard 100 µl of the contents from the last microwells (F1, F2) used.

Figure 2



In case of an **external standard dilution** (see 0), pipette 100 µl of these standard dilutions (S1 - S6) in the standard wells according to Table 1.

Table 1:

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



	1	2	3	4
<b>A</b>	Standard 1 (400.0 U/ml)	Standard 1 (400.0 U/ml)	Sample 2	Sample 2
<b>B</b>	Standard 2 (200.0 U/ml)	Standard 2 (200.0 U/ml)	Sample 3	Sample 3
<b>C</b>	Standard 3 (100.0 U/ml)	Standard 3 (100.0 U/ml)	Sample 4	Sample 4
<b>D</b>	Standard 4 (50.0 U/ml)	Standard 4 (50.0 U/ml)	Sample 5	Sample 5
<b>E</b>	Standard 5 (25.0 U/ml)	Standard 5 (25.0 U/ml)	Sample 6	Sample 6
<b>F</b>	Standard 6 (12.5 U/ml)	Standard 6 (12.5 U/ml)	Sample 7	Sample 7
<b>G</b>	Blank	Blank	Sample 8	Sample 8
<b>H</b>	Sample 1	Sample 1	Sample 9	Sample 9

- d. Add 100 µl of **Assay Buffer** in duplicate to the **blank wells**.
- e. Add 100 µl of each **sample** in duplicate to the **sample wells**.
- f. Prepare **Biotin-Conjugate** (see Preparation of Biotin-Conjugate 0).
- g. Add 50 µl of **Biotin-Conjugate** to all wells.
- h. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 100 rpm.
- i. Prepare **Streptavidin-HRP** (refer to Preparation of Streptavidin-HRP 0).
- j. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b. of the test protocol. Proceed immediately to the next step.
- k. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.
- l. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 100 rpm.
- m. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b. of the test protocol. Proceed immediately to the next step.
- n. Pipette 100 µl of **TMB Substrate Solution** to all wells.
- o. 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 0.6 – 0.65.



- 
- p. 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.
- q. Read absorbance of each microwell on a spectrophotometer 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.**

#### 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 sCD23 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 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 human sCD23 concentration.
- **If instructions in this protocol have been followed controls have been diluted 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human sCD23 levels (Hook Effect). Such samples require further external predilution according to expected human sCD23 values with Assay Buffer (1x) in order to precisely quantitate the actual human sCD23 level.**
- It is suggested that each testing facility establishes a control sample of known human sCD23 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 3. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3

Representative standard curve for human sCD23 ELISA. Human sCD23 was diluted in serial 2-fold steps in Assay Buffer (1x). Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

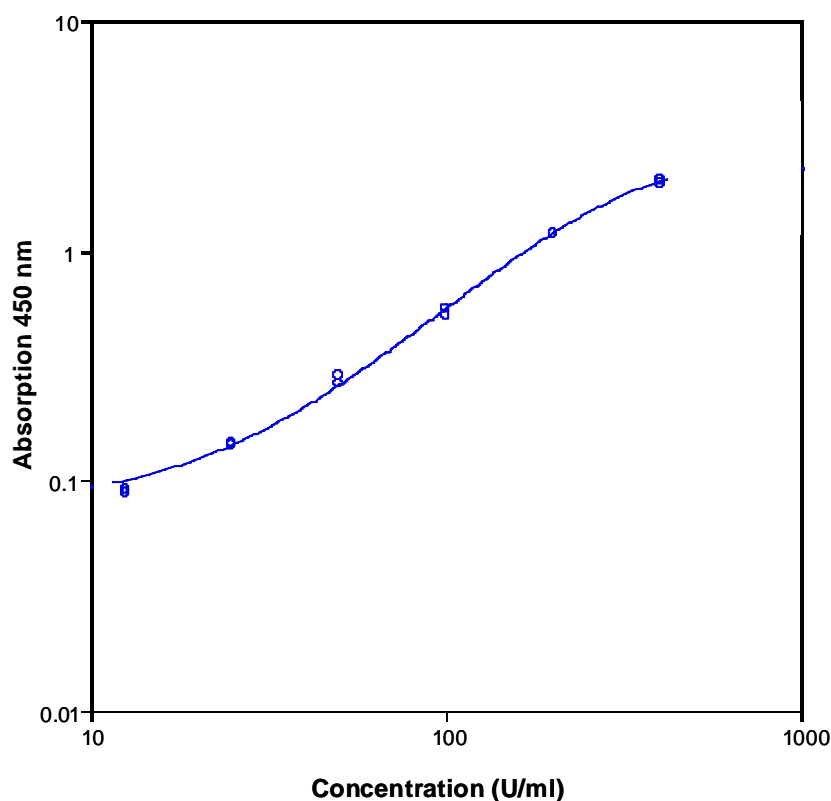


Table 2

Typical data using the human sCD23 ELISA

Measuring wavelength: 450 nm, Reference wavelength: 620 nm

Standard	Human sCD23 Concentration (U/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	400.0	1.964	1.984	1.4
	400.0	2.003		
2	200.0	1.190	1.191	0.1
	200.0	1.191		
3	100.0	0.528	0.543	3.8
	100.0	0.557		
4	50.0	0.265	0.276	5.6
	50.0	0.287		
5	25.0	0.143	0.145	1.5

Standard	Human sCD23 Concentration (U/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
	25.0	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. 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.

#### 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.

#### Performance Characteristics

##### Sensitivity

The limit of detection of human sCD23 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 6.8 U/ml (mean of 6 independent assays).

##### Reproducibility

###### 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 sCD23. 2 standard curves were run on each plate. Data below show the mean human sCD23 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 sCD23 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human sCD23 Concentration (U/ml)	Coefficient of Variation (%)
1	1	55.8	6.3
	2	51.1	1.6
	3	50.1	4.5
2	1	71.1	4.5
	2	65.3	2.7
	3	65.4	3.1
3	1	56.9	2.1
	2	53.4	2.7
	3	52.9	4.4
4	1	67.3	4.0
	2	63.4	1.1
	3	63.8	3.9
5	1	39.9	2.0
	2	38.3	10.9
	3	39.4	10.4
6	1	67.1	3.2
	2	62.9	3.4
	3	55.4	1.6
7	1	32.1	4.5
	2	29.7	1.6
	3	27.7	10.3
8	1	34.7	4.5
	2	30.6	1.9
	3	26.6	1.7

**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 sCD23. 2 standard curves were run on each plate. Data below show the mean human sCD23 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 6.3%.

Table 4: The mean human sCD23 concentration and the coefficient of variation of each sample

Sample	Mean Human sCD23 Concentration (U/ml)	Coefficient of Variation (%)
1	52.3	5.8
2	67.3	5.0
3	54.4	4.0
4	64.8	3.3
5	39.2	2.1
6	61.8	9.6
7	29.8	7.5
8	30.6	13.3

**Spike Recovery**

The spike recovery was evaluated by spiking 4 levels of human sCD23 into different pooled normal human serum samples. Recoveries were determined in 3 independent experiments with 4 replicates each.

The amount of endogenous human sCD23 in unspiked serum was subtracted from the spike values.

The recovery ranged from 81% to 120% with an overall mean recovery of 93%.

**Dilution Parallelism**

4 serum samples with different levels of human sCD23 were analysed at serial 2 fold dilutions with 4 replicates each.

The recovery ranged from 88% to 113% with an overall recovery of 100% (see Table 5).

Table 5

Sample	Dilution	Expected Human sCD23 Concentration (U/ml)	Observed Human sCD23 Concentration (U/ml)	Recovery of Expected Human sCD23 Concentration (%)
1	-	-	87.3	-
	1:2	43.6	40.4	93
	1:4	21.8	19.3	88
2	-	-	43.7	-
	1:2	21.9	20.2	93
	1:4	10.9	12.1	111
3	-	-	131.4	-
	1:2	65.7	66.3	101
	1:4	32.8	32.4	99
4	-	-	92.0	-
	1:2	46.0	46.4	101
	1:4	23.0	26.0	113

**Sample Stability****Freeze-Thaw Stability**

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

**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 sCD23 level determined after 24 h. There was no significant loss of human sCD23 immunoreactivity detected during storage under above conditions.

**Comparison of Serum and Plasma**

From 8 individuals, serum as well as EDTA, citrate and heparin plasma obtained at the same time point, was evaluated. All these blood preparations were found suitable for human sCD23 determinations, although human sCD23 values in citrate and EDTA plasma were slightly lower than serum values. It is, therefore, highly recommended to assure the uniformity of blood preparations.

**Specificity**

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human sCD23 positive serum.

There was no cross reactivity detected.

**Expected Values**

A panel of 8 serum samples from randomly selected apparently healthy donors (males and females) was tested for human sCD23.

The detected human sCD23 levels ranged between 10 and 91 U/ml with a mean level of 47 U/ml.

The levels measured may vary with the sample collection used.

**Bibliography**

1. Bengtsson M., et al. (1989). B-cell reconstitution after autologous bone marrow transplantation: increase in serum CD23 (IgE-binding factor) precedes IgE and B-cell regeneration. *Blood* 73, 2139-2144.
2. Sarfati D., et al. (1988). Possible role of human lymphocyte receptor for IgE (CD23) or its soluble fragments in the in vitro synthesis of human IgE. *J. Immunol.* 141, 2195-2199.
3. Cairn J., et al. (1988). Soluble CD23 is released by B lymphocytes cycling in response to IL-4 and anti-Bp50 (CDw40). *Eur. J. Immunol.* 18, 349-353.
4. Dadmarz R., et al. (1988). Heterogeneity of CLL: high CD23 antigen and aTNF receptor expression are features of favorable disease and of cell activation. *Br. J. Hematol.* 68, 279-282.
5. Genot E., et al. (1989). Effect of interferon- $\alpha$  on the expression and release of the CD23 molecule in hairy cell leukemia. *Blood* 74, 2455-2463.
6. Gordon J., et al. (1987). The molecules controlling B lymphocytes. *Immunol. Today* 8, 339-344.
7. Kikutani H., et al. (1986). Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell* 47, 657-665.

Revised 26 Aug. 2009 (Vers. 2.0)

8. Sarfati D., et al. (1988). Elevation of IgE-binding factors in serum of patients with B cell-derived chronic lymphocytic leukemia. Blood 71, 94-98.
9. Small T., et al. (1990). B-cell differentiation following autologous, conventional or T-cell depleted bone marrow transplantation: a recapitulation of normal B-cell ontogeny. Blood 76, 1647-1656.

## Reagent Preparation Summary

**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

**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

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

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

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

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

**Human sCD23 Standard**Reconstitute lyophilized **human sCD23 standard** with distilled water. (Reconstitution volume is stated on the label of the standard vial.)**Controls**Add 1000 µl distilled water to lyophilized **controls**. Predilute 1:2 in Assay Buffer (1x).













## Test Protocol Summary











1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add  $\mu$ l Assay Buffer (1x), in duplicate, to all standard wells leaving the first wells empty. Pipette 200  $\mu$ l prepared standard into the first wells and create standard dilutions by transferring 100  $\mu$ l from well to well. Discard 100  $\mu$ l from the last wells.  
Alternatively external standard dilution in tubes (see 0): Pipette 100  $\mu$ l of these standard dilutions in the microwell strips.
4. Add 100  $\mu$ l Assay Buffer (1x), in duplicate, to the blank wells.
5. Add 100  $\mu$ l sample in duplicate, to designated sample wells.
6. Prepare Biotin-Conjugate.
7. Add 50  $\mu$ l Biotin-Conjugate to all wells.
8. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
9. Prepare Streptavidin-HRP.
10. Empty and wash microwell strips 4 times with Wash Buffer.
11. Add 100  $\mu$ l diluted Streptavidin-HRP to all wells.
12. Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
13. Empty and wash microwell strips 4 times with Wash Buffer.
14. Add 100  $\mu$ l 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  $\mu$ l 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 controls have been diluted 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**

Revised 26 Aug. 2009 (Vers. 2.0)

## Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
	Conformidade com as normas europeias	Europæisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
				
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..