DRG® sCD-23 ELISA (EIA-3874)

Revised 15 June 2007 **RUO in the USA**

APPLICATION AND INTENDED USE

The low affinity Fc receptor for IgE (Fc e RII/CD-23) is expressed on various cell populations including B and T lymphocytes, monocytes, macrophages, eosinophils, platelets, and Langerhans Cells. Membrane CD-23 is an activation marker for B-lymphocytes involved in antigen presentation which expression is up regulated by IL-4 and down regulated by IFN-y. Proteolysis cleaves the CD-23 molecule from the cell membrane, giving rise to heterogenous bioactive fragments (sCD-23) involved in ß lymphocytes growth and differentiation IgE regulation, or in association with IL-1 in thymocyte differentiation. Therefore, sCD-23 may be considered as a novel cytokine with multiple functions. Increased serum levels of sCD-23 have been found in patients with ß-chronic lymphocyte leukaemia, bone marrow transplantation, allergy, inflammatory diseases, or autoimmune diseases.

PRINCIPLES OF THE DRG-sCD-23 EIA ASSAY

The DRG-sCD-23 ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiter plate. The assay is based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of sCD-23 are used. Antibody-producing cells are immortalized using the myeloma cell fusion method of Kohler and Milstein. A hybridoma cell is produced which secretes specific homogeneous antibodies. The use of a number of distinct MAbs avoids hyperspecificity and allows high sensitive assays with extended standard range and short incubation time. Standards or samples containing sCD-23 react with capture monoclonal antibodies (MAbs 1) coated on the microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich : coated MAbs 1 – sCD-23 - MAb 2 - HRP, the microtiter plate is washed to remove unbound enzyme labelled antibodies. Bound enzyme-labelled antibodies are measured through a chromogenic reaction. Chromogenic solution (TMB+H_2O_2) is added and incubated. The reaction is stopped with the addition of Stop Solution (H_2SO_4) and the microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance, which is proportional to the sCD-23 concentration. A standard curve is plotted and sCD-23 concentrations in a sample are determined by interpolation from the standard curve. The use of the ELISA Reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in high sensitivity in the low range and in an extended standard range.

REAGENTS PROVIDED

Reagents	96 tests kit	192 tests kit	Reconstitution
Microtiter plate with 96 anti-sCD-23 coated wells	96 wells	2 x 96 wells	Ready for use
Standards 0 to 5 in bovine serum and preservatives: see vial labels for exact concentrations	6 vials lyophil.	6 vials lyophil.	Add 1 ml distilled water
Anti-sCD-23-HRP Conjugate in buffered solution with proteins and preservatives	1 vial 6 ml.	2 vials 6 ml	Ready for use
Diluent (bovine serum with preservatives)	3 vials Lyophil	5 vials lyophil	Add distilled water (see the volume on the label)
Controls 1 and 2 in human plasma, with preservatives	2 vials lyophil.	2 vials lyophil.	Add 1 ml distilled water
Washing Solution Concentrate (buffer with preservatives)	1 vial 10 ml.	1 vial 10 ml	Dilute 2 ml in 400ml distilled water or the vial content in 2000 ml distilled water.
Concentred Chromogen : TMB (Tetrametylbenzidine) in DMF	1 vial 1 ml	1 vial 1 ml	Pipette 0.2 ml into 1 vial of Substrate Buffer
Substrate Buffer : H_2O_2 in acetate/citrate buffer	3 vials 21 ml	3 vials 21 ml	Ready for use
Stop Solution: H_2SO_4 1.8 N	1 vials 6 ml	1 vial 11 ml	Ready for use

Note: 1 U of the standard preparation is equivalent to 1.25ng/ml of sCD-23 (25kDa).

PRECAUTIONS AND WARNINGS

1. The human blood components included in this kit have been tested by European approved and USA FDA approved methods and found negative for HBsAg, anti-HCV and anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum, or plasma specimens should be in accordance with local safety procedures.
2. Avoid any skin contact with Stop Solution (H_2SO_4) and Concentrated Chromogen (TMB), Substrate Buffer, and Chromogenic Solution. In case of contact wash thoroughly with water.
3. Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
4. Do not pipet liquids by mouth.

EQUIPMENT AND SUPPLIES REQUIRED BUT NOT PROVIDED

1. High quality distilled water.
2. Precision pipette: 50 µl, 200 µl, 1 ml and 10 ml.
3. Vortex mixer and magnetic stirrer.
4. Horizontal microtiter plate shaker capable of 700 rpm ± 100 rpm, microtiter plate reader capable of reading at 450 nm and 490 nm, microtiter plate washer.

REAGENT PREPARATION

1. Standards, Controls and Diluent : Reconstitute the lyophilized Standards, Controls and diluent to the volume specified on the vial label with distilled water (1 ml for Standards and Controls). Allow them to remain undisturbed until completely dissolved, and then mix well by gentle inversion.
2. Wash Solution: Dilute 2 ml of Washing Solution Concentrate in 400 ml distilled water or all the contents of the Washing Solution Concentrate vial in 2000 ml distilled water (use a magnetic stirrer).
3. Chromogenic Solution: pipette 0.2 ml of the Concentrated Chromogen (TMB) into one of the vials of Substrate Buffer (H_2O_2 in acetate/citrate buffer). Extemporaneous preparation is necessary. Use only at room temperature. Chromogenic Solution is stable for a maximum of 15 min. Avoid direct exposure to sunlight (see section X.A.11.).

STORAGE AND SHELF LIFE OF REAGENTS

A. UNOPENED vials

Store the unopened vials at 2°C to 8°C. All kit components are stable until the expiry date printed on the labels.

B. OPENED vials

1. The Conjugate vial must be stored at 2° to 8°C.
2. The reconstituted Standards, Controls and Diluent are stable for 4 days at 2°C to 8°C. Aliquot held for longer periods of time should be frozen, a maximum of two times, at –20°C (maximum 2 months) or at –70°C for longer storage (until expiration date).
3. Store the unused strips at 2°C to 8°C in the sealed bag containing the desiccant until expiration date.
4. The Wash Solution Concentrate is stable at room temperature until expiration date. In order to avoid washerhead obstructions, it is recommended to prepare a fresh diluted Wash Solution each day.
5. The freshly prepared Chromogenic Solution is stable for a maximum of 15 min. at room temperature and must be discarded afterwards.

SPECIMEN COLLECTION, PREPARATION, STORAGE AND DILUTION

A. Specimen Collection and preparation

1. The sCd-23 ELISA kit may be used to measure sCD-23 in serum, plasma, cell culture supernatant as well as other biological fluids. Isolation and culture of peripheral blood mononuclear cells may be realized by usual methods. However, one should avoid an unintentional stimulation of the cells by the procedure. The use of pyrogen-free reagents and adequate controls are mandatory.
2. Sampling conditions can affect values measured in serum or plasma, therefore, strict precautions have to be taken during sampling to avoid impurities contained in sampling materials that would stimulate sCD-23 production by blood cells and thus falsely increase plasma sCD-23 values.
3. Serum must be removed as soon as possible from the clot of red cells after clotting and centrifugation, and kept at 4°C.
4. Collection tubes must be pyrogen-free. Plasma can be collected on sterile EDTA or heparin tubes (at 4°C) and rapidly separated after centrifugation. However, as batches of heparin are often contaminated with pyrogen, it is recommended to test each batch of heparin to avoid unintentional stimulation of blood cells. Other substances in the tube must be also pyrogen-free.
5. These recommendations are also valuable for other biological fluids (cell culture supernatant, etc.).

B. Storage

Serum/plasma samples must be kept at –20°C for maximum 2 months, and for longer storage (maximum one year) at – 70°C. Samples with low protein levels (e.g. cell culture medium, urine, etc.) should be stored at –70°C (maximum one year).

C. Sample Dilution

If samples generate values higher than the highest standard, dilute the sample with the appropriate solution (see below) and repeat the assay.

1. **Serum and plasma: dilute with diluent.**
2. **Cell culture supernatant and urine**:
   a) High concentration expected (> 2,5u/ml)
      Dilute at least 1/4 the samples with diluent
   b) Low concentration expected (< 2,5u/ml)
      Reconstitute the Special Pt 0 Receptor vials with the samples (no dilution).
      R. / Each Special Pt0 Receptor vial must be reconstituted by one sample.

sCD-23 EIA PROCEDURE

The instructions of the assay procedure must be followed to obtain reliable results.

A. Procedural notes

1. Allow the samples and reagents to equilibrate to room temperature (18°C to 25°C) before commencing the assay. Thoroughly mix the reagents and samples before use by gentle agitation or swirling.
2. Do not use kit components beyond the expiration date.
3. Do not mix materials from different kit lots.
4. Do not mix strips from different plates.
5. Perform Standards,Controls and Unknowns in duplicate. Vertical alignment is recommended.
6. A standard curve should be run with each assay run or each plate run.
7. To avoid drift, the time between pipetting of the first standard and the last sample must be no longer than 30 minutes. Otherwise, results will be affected.

8. Use a clean disposable plastic pipette for each reagent, standard, control or specimen addition in order to avoid cross contamination.
9. For the dispensing of the Chromogenic Solution and Stop Solution avoid pipettes with metal parts.
10. Use a clean plastic container to prepare the Wash Solution.
11. The Chromogenic Solution should be colourless. If a blue colour develops within a few minutes after preparation, this indicates that the reagent is unusable, and must be discarded. Dispense the Chromogenic Solution within 15 min. following the washing of the microtiter plate.
12. During incubation with Chromogenic Solution, avoid direct sunlight on the microtiter plate.
13. Respect the incubation times described in the assay procedure.

B. Assay Procedure

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 100 µl of Diluent into all wells.
4. Pipette 100 µl of each Standard, Control, or Sample into the appropriate wells.
5. Pipette 50µl of anti-sCD-23 Conjugate into all the wells.
6. Incubate for 2 hours at room temperature on a horizontal shaker set at 700-rpm ± 100 rpm.
7. Aspirate the liquid from each well; wash the plate three times by:
    a) Dispensing of 0.4 ml of Medgenix Wash Solution into each well.
    b) Aspirating the content of each well.
8. Pipette 200 µl of freshly prepared Chromogenic Solution into each well within 15 min. following the washing step.
9. Incubate the plate for 30 min. at room temperature on an horizontal shaker set at 700 ± 100 rpm, avoiding direct sunlight.
10. Pipette 50 µl of Stop Solution into each well. Read absorbances at 450 nm and 490 nm (reference filter: 630 or 650 nm) within 3 hours and calculate the results.

CALCULATION OF ANALYTICAL RESULTS:

A. Reading the plate with the DRG ELISA Reader

Read the plate according to the instructions of the DRG ELISA Reader and ELISA Software.

B Reading the plate with other equipment

Read the microtiter plate at 450 nm (reference filter: 630 or 650 nm).

- Construct a standard curve using all standard points for which absorbances are below the limit of linearity of reader used.
- Plot the OD on the ordinate against the standard concentrations on the abscissa using either linear or semi-log graph paper and draw the curve by connecting the plotted points with straight lines.
- Determine sCD-23 concentrations of Samples or Controls for which absorbance is no greater than those of the last standard plotted at 450 nm.
- If any Control or Sample has an absorbance greater than the absorbance of the last standard read at 450 nm, a second reading at 490 nm (reference filter: 630 or 650 nm) is needed. Proceed as described above to construct a second standard curve at 490 nm using all the standard points.
- The segment of the curve drawn between the last standard read at 450 nm and the most concentrate standard will be considered at 490 nm. The concentration of Samples and Controls for which absorbance is included in this segment, is read at 490 nm. So, the first reading gives the high sensitivity of the assay and the second reading allows an extended standard range.

Note: The readings at 490 nm are only for off-scale values at 450 nm (above the limit of reader linearity) and should not replace the reading at 450 nm for values below the limit of reader linearity.

C. Example of a typical reference curve

The following data are for demonstration purpose only and cannot be used in place of data generated at the time of assay.

sCD-23 EIA		Polychromatic model (OD units)
Standard	0 U/ml	0.083
	1 U/ml	0.149
	2,5 U/ml	0.309
	5 U/ml	0,626
	10 U/ml	1.390
	20 U/ml	2,918

QUALITY CONTROL

The two controls provided in the kit can be used as internal laboratory controls. **Note:** Other controls, which contain azide, will interfere with the enzymatic reaction and cannot be used. Serum or heparin plasma pools as well as stimulated cell culture supernatant can be collected and frozen immediately in aliquot to serve as controls. Repeated freezing and thawing are not permitted.

RECORD KEEPING

It is good laboratory practice to record the kit lot numbers and date of reconstitution for the reagents in use.

CONTROLS

It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. It is recommended that quality controls charts be maintained to monitor the performance of the kits. Control ranges are indicated on vial labels. Out of range control results indicate the assay must be repeated. Repeat patient samples may also be used to measure interassay precision.

SAMPLE HANDLING

Strictly adhere to the instruction for handling and storage of samples. Standards, Controls, and Unknowns should be run in duplicate. A clean disposable tip should always be used to avoid carryover contamination.

DATA REDUCTION

It is good practice to construct a standard curve for each run to check visually the curve fit selected by the computer program.

EXPECTED RANGE (Reference Interval)

At the present stage of our studies, only preliminary results can be provided and we thus recommend that each laboratory establish its own normal values. For guidance, the mean of 118 normal plasma was 1.3U/ml (SD =0,6), ranging between 0,15 U/ml and 3,3U/ml. This study was performed with samples collected in strict sampling condition.

PERFORMANCE CHARACTERISTICS

MINIMUM DETECTABLE CONCENTRATION (MDC).

The MDC is estimated to be 0,15 U/ml and is defined as the sCD-23 concentration corresponding to the average OD of 20 replicates of the zero standard + 2 standard deviations.

PRECISION

INTRA-ASSAY				INTER-ASSAY (day-to-day)			
Sample	n	<X> ± SD (U/ml)	CV %	Sample	n	<X> ± SD (U/ml)	CV %
Serum 1	20	2,4 ± 0,08	3.3	Serum 1	20	2,0 ± 0,08	4,0
2	20	14,0 ± 0,44	3,1	2	20	10,0 ± 0,43	4,3

BIOCHEMICAL CHARACTERISATION

The DRG sCD-23 recognizes the 15, 25, 29 and 37 kDa fragments of recombinant sCD-23. 1U DRG corresponds to 1,25 ng/ml of r-sCD-23 (recombinant 25 kDa form).

ACCURACY

	RECOVERY			DILUTION TEST				
Sample	Added sCD-23 (U/ml)	Recovery GM-CSF (U/ml)	Recovery (%)	Sample	Dilution	Theor. conc. (U/ml)	Meas. conc. (U/ml)	Recovery (%)
Serum	13,7	12,5	91	Serum	1/1 1/2 1/4 1/8	**8,6** **4,3** **2,15** **1,07**	**8,6** **4** **2** **1**	100 93 93 93
Cell Culture Medium 1	13,7	13,3	97	Serum	1/1 1/2 1/4	**4,4** **2,2** **1,1**	**4,4** **2,1** **1,1**	100 95 100
Cell Culture Medium 2	13,7	13,7	100	Cell Culture Medium 1	1/1 1/2 1/4 1/8	**15,4** **7,7** **3,85** **1,92**	**15,4** **7,5** **3,62** **1,85**	100 97 94 96
				Cell Culture Medium 2	1/1 1/2 1/4	3,0 1,5 0,75	3 1,5 0,77	100 100 103

HIGH DOSE HOOK-EFFECT

A sample spiked with sCD-23 up to 365 U/ml gives a response higher than that obtained for the last standard point.

LITERATURE REFERENCES

1 J. Cordon et. Al (1989) CD-23: a multi-functional receptor/ lymphokine? Immun. Today 10,5
2. G Delespesse et. Al. (1989) Human IgE- binding factors Immun. Today 10,5
3. J. Cordon et. Al (1991) CD-23: a multi-functional regulation of the immune system that binds IgE Monograph in Allergy, Ed. Karger (Basel)
4. B. Descamps- Latscha et. Al. (1993) Soluble CD-23 as an affector of immune dysregulation in chronic uremia and dialysis Kidney Intern, 43; 878-884

SUMMARY OF ASSAY PROCEDURE

	Standards (µl)	Controls-samples (µl)
Diluent **Standards (0-5) Controls Serum/plasma samples**	100 100 -	100 - 100
Anti-sCD-23 Conjugate	50	50
Incubate for 2 hours at R.T. with continuous shaking (700 RPM) **Aspirate** the contents of each well **Wash** 3 times with 0.4 ml of Wash Solution and aspirate		
Chromogenic Solution	200	200
Incubate 30 min. at R.T. with continuous shaking (700 RPM)		
Stop Solution	50	50
Read on a microtiter plate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm) and 490 nm (versus 630 or 650 nm).		