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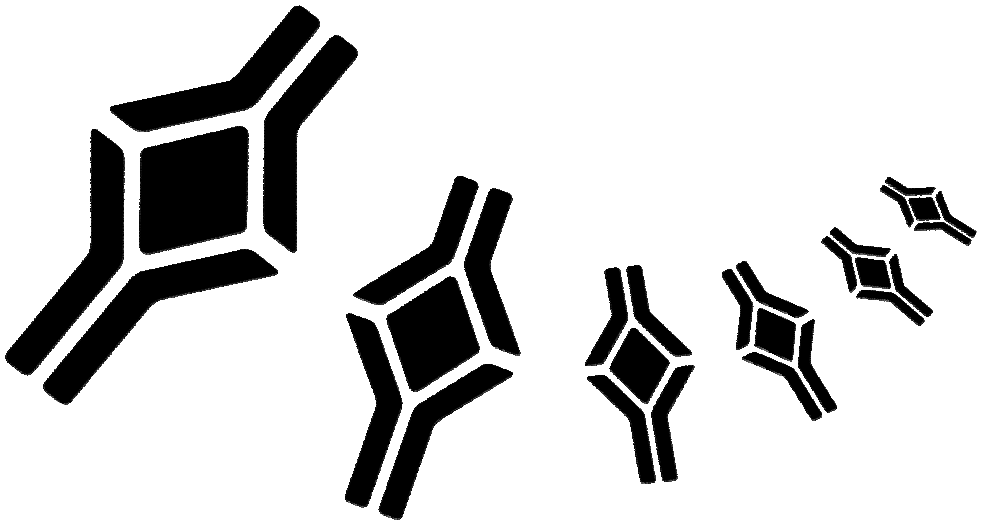


# Human sCD153 (sCD30L) ELISA

Product Data Sheet

Cat. No.: RBMS298R

For Research Use Only



VERSION 51 040708

## 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

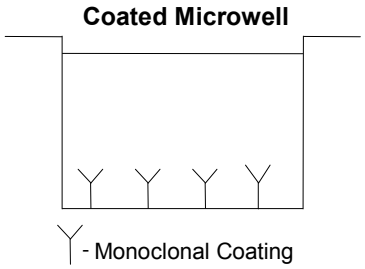
The sCD153 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human sCD153 in cell culture supernatants, human serum, plasma or other body fluids. **The sCD153 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

# 2 SUMMARY

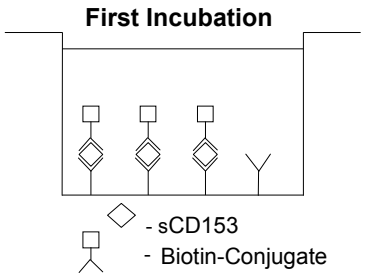
The human CD30 ligand, CD153, is a 40kDa Type II transmembrane glycoprotein. It interacts with its receptor, CD30, as a homotrimer (1) Soluble trimeric CD153 binds to membrane-anchored CD30 with a relatively high affinity and is very effective in triggering cell death and TNFα (4). CD153 is a member of the TNF superfamily which is primarily expressed by activated T cells, B cells and monocytes (2, 3, 5, 6). Its function in programmed cell death has been proven, as well as its involvement in disorders like a variety of lymphoid malignancies like Hodgkin's disease and anaplastic large cell lymphoma.

# 3 PRINCIPLES OF THE TEST

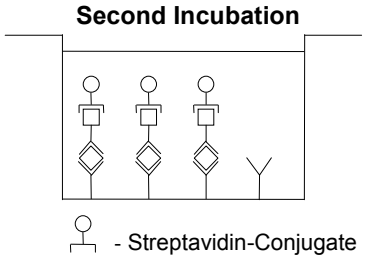
An anti-sCD153 monoclonal coating antibody is adsorbed onto microwells.




sCD153 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-sCD153 antibody is added and binds to sCD153 captured by the first antibody.




Following incubation unbound biotin conjugated anti-sCD153 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-sCD153. 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 sCD153

1 vial (100 µl) **Biotin-Conjugate** anti-sCD153 monoclonal antibody

1 vial (150 µl) **Streptavidin-HRP**

2 vials **sCD153 Standard**, lyophilized, 200 ng/ml upon reconstitution

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 and 10 % BSA)

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

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

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 sCD153. 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

- 10 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 research use only and are not for use in diagnostic or therapeutic procedures.

- Do not mix or substitute reagents with those from other lots or other sources.

- Do not use kit reagents beyond expiration date on label.

- Do not expose kit reagents to strong light during storage or incubation.

- Do not pipette by mouth.

- Do not eat or smoke in areas where kit reagents or samples are handled.

- Avoid contact of skin or mucous membranes with kit reagents or specimens.

- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.

- Avoid contact of substrate solution with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate 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. 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 (5.0 ml) to 95 ml 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 sCD153 Standard

Reconstitute sCD153 **Standard** by addition of distilled water. Reconstitution volume is stated on the label. 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 | 0.06 | 5.94 |
| 1 - 12 | 0.12 | 11.88 |

## F. 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*** |

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 sCD153 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.) **sCD153 Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl 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 sCD153 standard dilutions ranging from 100 to 1.56 ng/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of sCD153 standard dilutions:

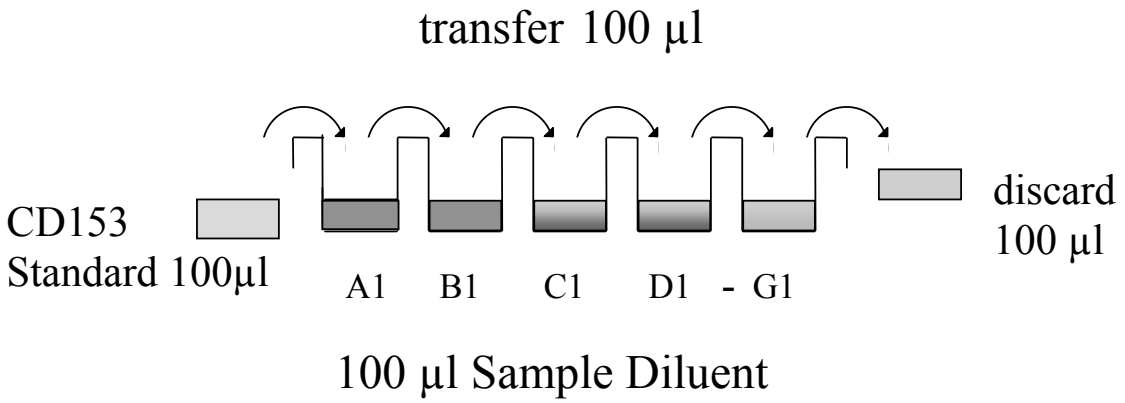



Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **A** | Standard 1 (100 ng/ml) | Standard 1 (100 ng/ml) | Sample 1 | Sample 1 |
| **B** | Standard 2 (50 ng/ml) | Standard 2 (50 ng/ml) | Sample 2 | Sample 2 |
| **C** | Standard 3 (25 ng/ml) | Standard 3 (25 ng/ml) | Sample 3 | Sample 3 |
| **D** | Standard 4 (12.5 ng/ml) | Standard 4 (12.5 ng/ml) | Sample 4 | Sample 4 |
| **E** | Standard 5 (6.25 ng/ml) | Standard 5 (6.25 ng/ml) | Sample 5 | Sample 5 |
| **F** | Standard 6 (3.13 ng/ml) | Standard 6 (3.13 ng/ml) | Sample 6 | Sample 6 |
| **G** | Standard 7 (1.56 ng/ml) | Standard 7 (1.56 ng/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 50 µl of **Sample Diluent**, in duplicate, to the sample wells.

g. Add 50 µl of each **Sample**, in duplicate, to the designated wells.

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

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 9.E).

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. 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.

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

q. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.
**The colour development on the plate should be monitored and the substrate reaction stopped (see point r. 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 620nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.

r. 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.

s. 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 sCD153 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 sCD153 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating sCD153 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 sCD153 concentration.

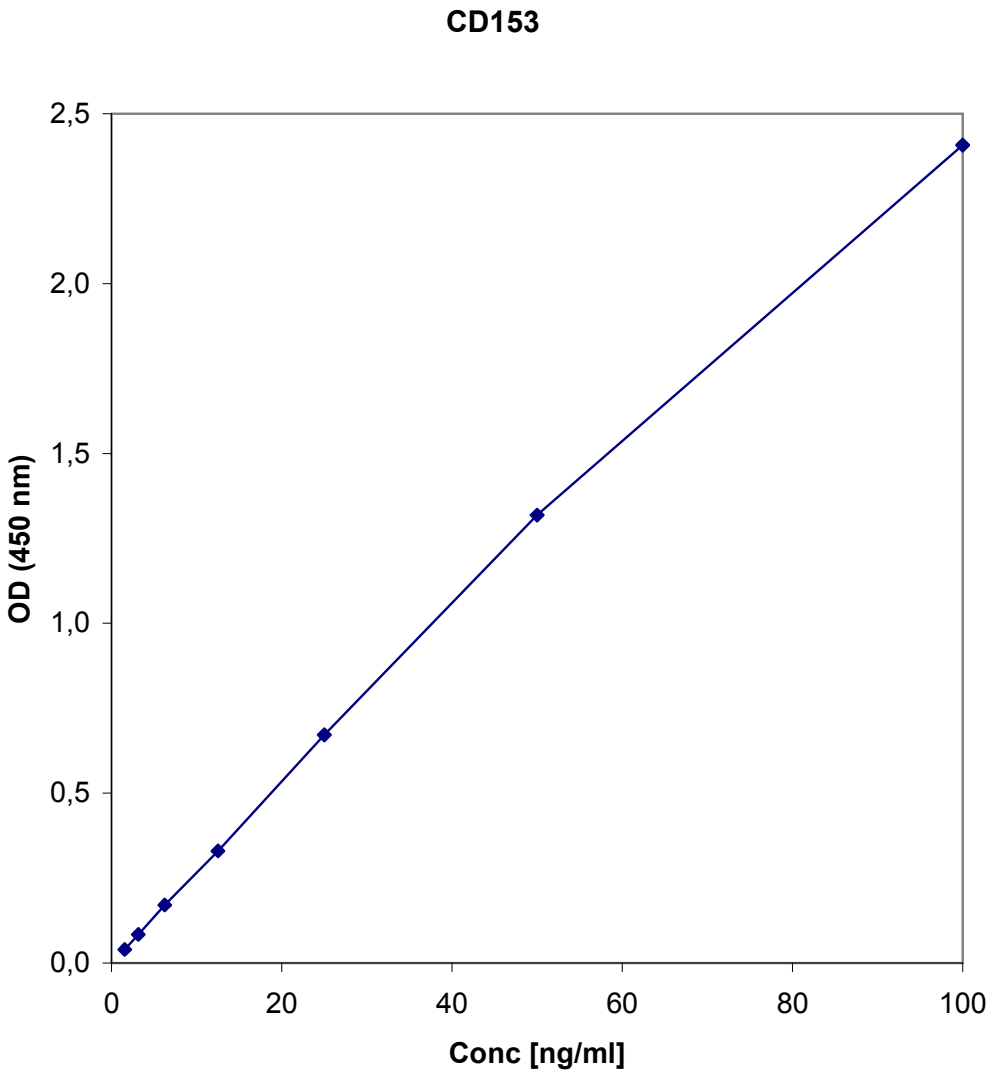
  **For samples which have been diluted according to the instructions given in this manual 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2).**

  **Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD153 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual sCD153 level.**

  It is suggested that each testing facility establishes a control sample of known sCD153 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 sCD153 ELISA. sCD153 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 sCD153 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

| Standard | sCD153 Concentration (ng/ml) | O.D. (450 nm) | O.D. Mean | CV % |
|---|---|---|---|---|
| 1 | 100 | 2.513 | 2.454 | 2.4 |
| | 100 | 2.396 | | |
| 2 | 50 | 1.403 | 1.365 | 2.8 |
| | 50 | 1.327 | | |
| 3 | 25 | 0.726 | 0.719 | 1.0 |
| | 25 | 0.711 | | |
| 4 | 12.5 | 0.365 | 0.377 | 3.1 |
| | 12.5 | 0.388 | | |
| 5 | 6.25 | 0.216 | 0.217 | 0.5 |
| | 6.25 | 0.218 | | |
| 6 | 3.13 | 0.138 | 0.131 | 5.7 |
| | 3.13 | 0.123 | | |
| 7 | 1.56 | 0.085 | 0.086 | 1.3 |
| | 1.56 | 0.088 | | |
| Blank | 0 | 0.049 | 0.049 | 0.0 |
| | 0 | 0.049 | | |

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 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 sCD153 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 0.5 ng/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 sCD153. Two standard curves were run on each plate. Data below show the mean sCD153 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 8.7%.

| Positive Sample | Experiment | sCD153 Concentration (ng/ml) | Coefficient of Variation (%) |
|---|---|---|---|
| 1 | 1 | 156.0 | 7 |
| | 2 | 125.3 | 9 |
| | 3 | 151.5 | 11 |
| 2 | 1 | 83.9 | 6 |
| | 2 | 91.0 | 5 |
| | 3 | 74.4 | 6 |
| 3 | 1 | 44.5 | 7 |
| | 2 | 49.1 | 8 |
| | 3 | 48.8 | 7 |
| 4 | 1 | 20.6 | 12 |
| | 2 | 26.6 | 5 |
| | 3 | 20.2 | 12 |
| 5 | 1 | 40.9 | 7 |
| | 2 | 42.7 | 9 |
| | 3 | 38.4 | 10 |
| 6 | 1 | 18.7 | 9 |
| | 2 | 21.2 | 9 |
| | 3 | 18.9 | 10 |
| 7 | 1 | 14.1 | 13 |
| | 2 | 13.9 | 12 |
| | 3 | 12.2 | 5 |
| 8 | 1 | 7.7 | 11 |
| | 2 | 6.8 | 11 |
| | 3 | 5.6 | 10 |

**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 sCD153. Two standard curves were run on each plate. Data below show the mean sCD153 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 8.7%.

| Sample | sCD153 Concentration (ng/ml) | Coefficient of Variation (%) |
|---|---|---|
| 1 | 154.9 | 2.0 |
| 2 | 83.1 | 10.0 |
| 3 | 47.5 | 5.5 |
| 4 | 22.5 | 15.8 |
| 5 | 40.7 | 5.4 |
| 6 | 19.6 | 7.1 |
| 7 | 13.4 | 7.8 |
| 8 | 6.7 | 16.1 |

## C. Spiking Recovery

The spiking recovery was evaluated by spiking four levels of sCD153 into 4 different pooled normal human sera. The amount of endogenous sCD153 in unspiked serum was substracted from the spike values. Mean recovery was 82%.

## D. Dilution Linearity

Four serum samples with different levels of sCD153 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 85% to 127% with an overall mean recovery of 105%.

| Sample | Dilution | sCD153 Concentration (pg/ml) Expected Value | Observed Value | % Recovery of Exp. Value |
|---|---|---|---|---|
| 1 | 1:2 | -- | 171.5 | -- |
| | 1:4 | 85.7 | 85.1 | 99.3 |
| | 1:8 | 42.6 | 42.2 | 99.1 |
| | 1:16 | 21.1 | 19.7 | 93.7 |
| 2 | 1:2 | -- | 95.1 | -- |
| | 1:4 | 47.6 | 40.2 | 84.5 |
| | 1:8 | 20.1 | 17.8 | 88.4 |
| | 1:16 | 8.9 | 7.8 | 87.4 |
| 3 | 1:2 | -- | 67.0 | -- |
| | 1:4 | 33.5 | 42.4 | 126.7 |
| | 1:8 | 21.2 | 24.2 | 114.0 |
| | 1:16 | 12.1 | 14.3 | 117.0 |
| 4 | 1:2 | -- | 33.2 | -- |
| | 1:4 | 16.6 | 20.3 | 122.7 |
| | 1:8 | 10.2 | 10.8 | 106.5 |
| | 1:16 | 5.4 | 6.4 | 118.9 |

## 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 sCD153 levels determined. There was no significant loss of sCD153 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 sCD153 level determined after 24 h. There was no significant loss of sCD153 immunoreactivity during storage at –20°C and 2-8°C, but about 20% loss of sCD153 immunoreactivity was seen following storage at RT and 40% at 37°C.

## F. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a sCD153 positive serum. There was no detectable cross reactivity.

## G. Expected Serum Values

There are no detectable sCD153 levels found in healthy blood donors.

# 14 REFERENCES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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 distilled water to each vial of lyophilized **sCD153 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 |

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells
- Pipette 100 µl reconstituted **sCD153 Standard** into the first wells and create standard dilutions ranging from 100 to 1.56 ng/ml by trans-ferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 50 µl **Sample Diluent** to the sample wells
- Add 50 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 3 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 a microplate shaker
- Empty and wash microwell strips 3 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 sCD153 levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual sCD153 level.**

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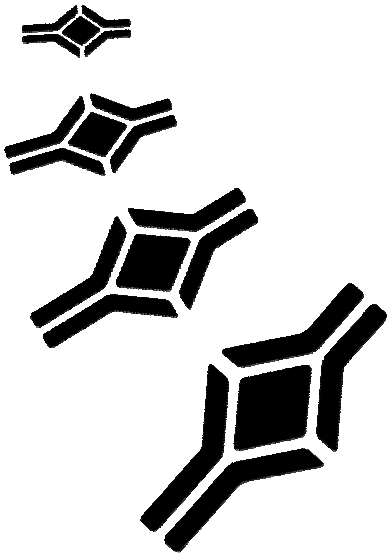


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