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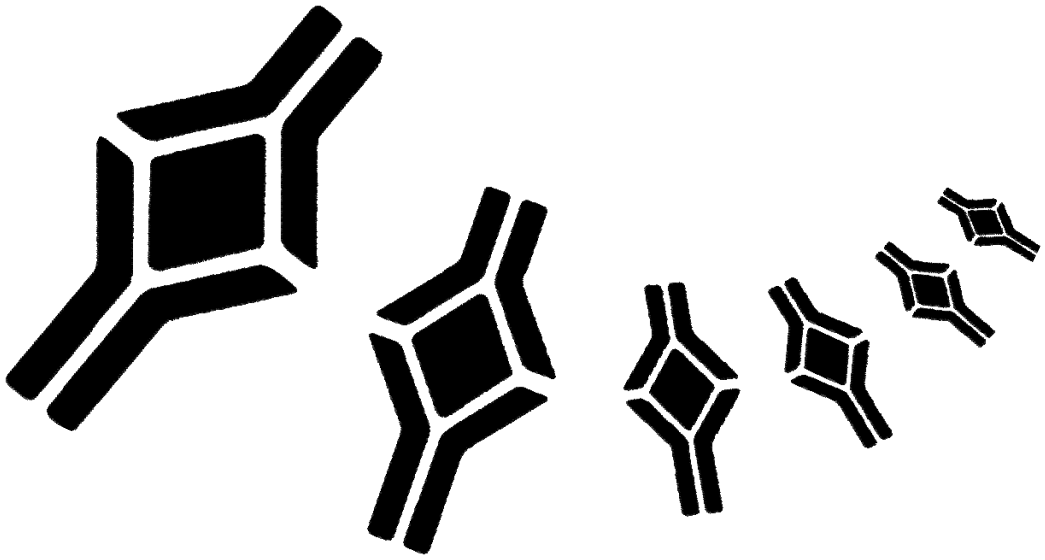


HUMAN SCD8 ELISA

Product Data Sheet

Cat. No.: RBMS226/2R

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 sCD8 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human sCD8 in cell culture supernatants, human serum, plasma or other body fluids. **The sCD8 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2. SUMMARY

CD8 is an cell surface antigen that belongs to the immunoglobulin superfamily (1). It occurs either as a disulfide-linked homodimer or homomultimer of 32kDA subunits or as a heterodimer complexed with CD8ß (2).

Human T lymphocytes are classified as helper-inducer (CD4) and suppressor-cytotoxic (CD8) according to the cell surface phenotypes (3).

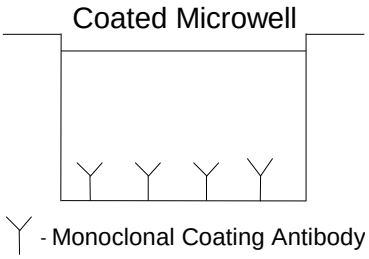
CD8 is crucial to the positive selection of cytotoxic T cells in the thymus (4).
It binds to class I MHC molecules and by this acts as an coreceptor and stabilizes the interaction of the T-cell antigen receptor with major histocompatibility complex (5).

An soluble form of CD8 has been described that seems to result from alternative mRNA splicing (6, 7).

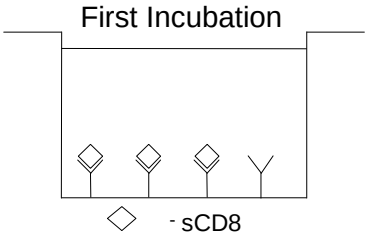
Serum levels of sCD8 have e.g. been found to be of importance in patients with the following diseases: leukemia (8), allograft transplantation (9, 10), as a marker for T-cell activation in HIV (11, 12, 13, 14), Graves' and Hashimoto's diseases (15), Multiple Sclerosis (16), depression (17), diabetes (18), and infectious mononucleosis (19).

3. PRINCIPLES OF THE TEST

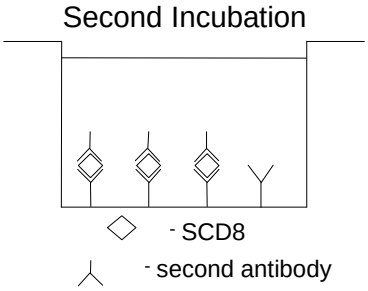
An anti-sCD8 monoclonal coating antibody is adsorbed onto microwells.



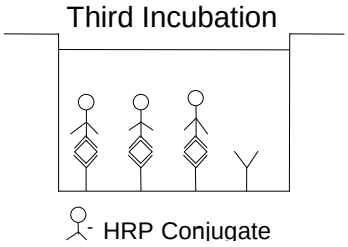
sCD8 present in the sample or standard binds to antibodies adsorbed to the microwells.

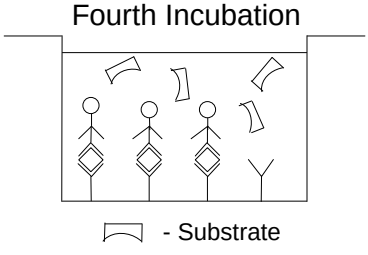


Following incubation unbound sCD8 is removed during a wash step. A polyclonal anti-sCD8 antibody is added and binds to sCD8 captured by the first antibody.

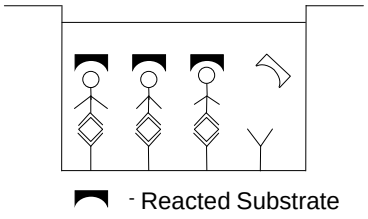


Following incubation unbound second anti-sCD8 is removed during a wash step. Anti-rabbit-HRP is added and binds to the second anti-sCD8. Following incubation unbound anti-rabbit-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.





A coloured product is formed in proportion to the amount of sCD8 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven sCD8 standard dilutions and sCD8 sample concentration determined.



4. REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with monoclonal Antibody (murine) to human sCD8

1 vial (150 µl) **Detection** anti-sCD8 polyclonal **Antibody** (rabbit)

1 vial (15 µl) **anti-rabbit-HRP**[1)]

2 vials **sCD8 Standard**[1)]**,** concentrate

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**

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

1 vial (0.4 ml) ***Blue-Dye***

1 vial (0.4 ml) ***Red-Dye***

1 vial (0.4 ml) ***Green-Dye***

6 adhesive **Plate Covers**

1) It is recommended to spin vial in microcentrifuge before use to collect reagent at the bottom.

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 sCD8. 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 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 Detection Antibody

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

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

D. Preparation of sCD8 Standard

Dilute sCD8 **Standard** by addition of **Assay Buffer**. Dilution volume is stated on the label of the standard vial. Mix gently to insure complete homogeneity.

E. Preparation of anti-rabbit-HRP

Add 135 µl **Assay Buffer** to the vial containing the concentrate antibody.
Make a 1:100 dilution of this predilution in **Assay Buffer** as needed according to the following table:

Number of Strips	Anti-rabbit-HRP (predilution) (ml)	Assay Buffer (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.8

Store predilution at 2-8°C.

F. Addition of Colour-giving Dyes

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. Detection Antibody: Before dilution of the concentrated detection antibody, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of detection antibody.

3 ml Assay Buffer	30 µl ***Green-Dye***
6 ml Assay Buffer	60 µl ***Green-Dye***

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

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

10. TEST PROTOCOL

a. Prepare reagents immediately before use and mix them thoroughly without foaming.

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** against human sCD8 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 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.

d. 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**.

d. Add 100 µl of **Assay Buffer** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of prediluted (Refer to preparation of reagents) **sCD8 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 sCD8 standard dilutions ranging from 2000 to 31.25 U/ml.
Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of sCD8 standard dilutions:

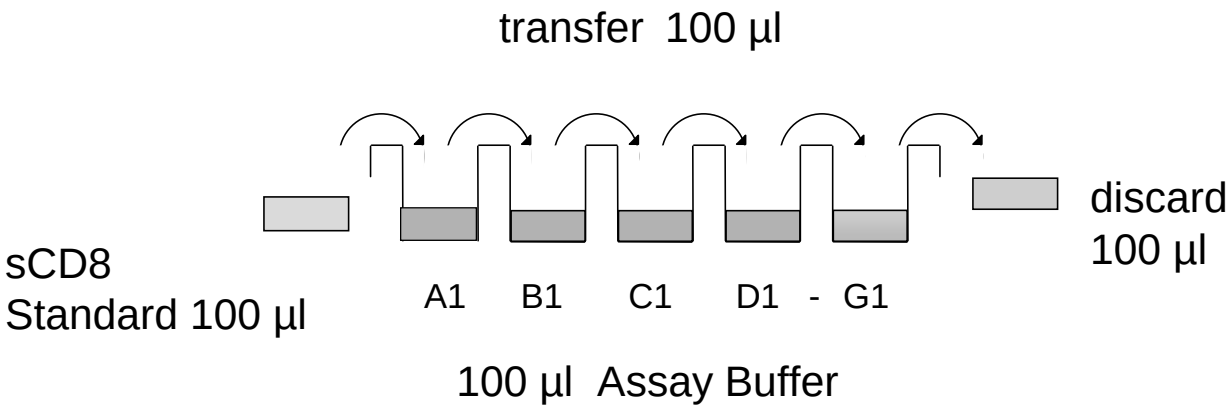


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (2000 U/ml)	Standard 1 (2000 U/ml)	Sample 1	Sample 1
B	Standard 2 (1000 U/ml)	Standard 2 (1000 U/ml)	Sample 2	Sample 2
C	Standard 3 (500 U/ml)	Standard 3 (500 U/ml)	Sample 3	Sample 3
D	Standard 4 (250 U/ml)	Standard 4 (250 U/ml)	Sample 4	Sample 4
E	Standard 5 (125 U/ml)	Standard 5 (125 U/ml)	Sample 5	Sample 5
F	Standard 6 (62.5 U/ml)	Standard 6 (62.5 U/ml)	Sample 6	Sample 6
G	Standard 7 (31.25 U/ml)	Standard 7 (31.25 U/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

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

f. Add 50 µl of **Assay Buffer** to the sample wells.

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

h. 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

i. Prepare **Detection Antibody**. (Refer to preparation of reagents)

j. 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.

k. Add 100 µl of diluted **Detection Antibody** to all wells, including the blank wells.

l. 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.

m. 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.

n. Prepare **anti-rabbit-HRP**. (Refer to preparation of reagents)

o. Add 100 µl of diluted **anti-rabbit-HRP** to all wells, including the blank wells.

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

q. 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.

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

s. 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 t. 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.

t. 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.

u. 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 sCD8 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 sCD8 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating sCD8 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 sCD8 concentration.

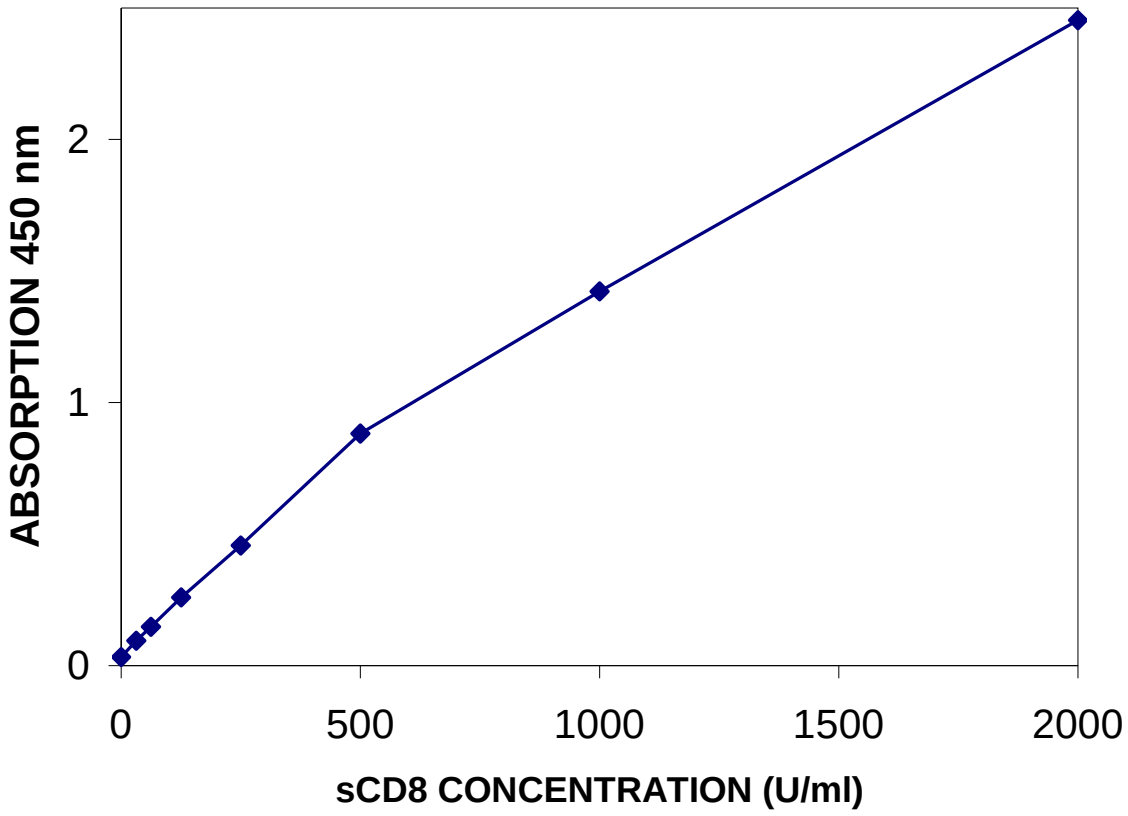
  If samples have been diluted according to the instructions given in this manual (e.g. cell culture supernatants), the concentration read from the standard curve must be multiplied by the respective dilution factor.

  Note: Calculation of samples with an O.D. exceeding standard 1 may result in incorrect sCD8 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual sCD8 level.

  It is suggested that each testing facility establishes a control sample of known sCD8 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 sCD8 ELISA. sCD8 was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of four 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 sCD8 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	sCD8 Concentration (U/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	2000	2.497	2.453	1.8%
	2000	2.409		
2	1000	1.401	1.423	1.5%
	1000	1.445		
3	500	0.874	0.8815	0.9%
	500	0.889		
4	250	0.451	0.457	1.3%
	250	0.463		
5	125	0.255	0.2595	1.7%
	125	0.264		
6	62.5	0.149	0.147	1.4%
	62.5	0.145		
7	31.25	0.09	0.0945	4.8%
	31.25	0.099		
Blank		0.032	0.033	
		0.034		

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.

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 sCD8 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 6.9 U/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in independent experiments. The overall intra-assay coefficient of variation has been calculated to be 6.1%.

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in independent experiments by two technicians. The overall inter-assay coefficient of variation has been calculated to be 5.5%.

C. Spiking Recovery

The spiking recovery was evaluated by spiking four levels of sCD8 into four human sera. Recoveries were determined in two independent experiments with 4 replicates each. Observed values showed an overall mean recovery of 81%.

D. Dilution Linearity

Human serum with different levels of sCD8 was assayed at four serial threefold dilutions with 4 replicates each. Experiments showed an overall mean recovery of 96.9 %.

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum were stored frozen at –20°C and thawed up to 3 times, and sCD8 levels determined. There was no significant loss of sCD8 by freezing and thawing up to 3 times.

b. Storage Stability

Aliquots of spiked serum were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the sCD8 level determined after
24 h. There was no significant loss of sCD8 immunoreactivity during storage at -20°C, 4°C , room temperature, and 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 sCD8 positive serum. There was no detectable cross reactivity.

G. Expected Serum Values

A panel of 16 sera from apparently healthy blood donors (male and female) was tested for sCD8. The detected sCD8 levels ranged between 144 and 767 U/ml with a mean level of 413 U/ml. The normal range may vary with the sample collective used.

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. Detection Antibody Make a 1:100 dilution according to the table.

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

D. Standard Dilute sCD8 **Standard** by addition of **Assay Buffer**. Dilution volume is stated on the label of the standard vial.

E. Anti-rabbit-HRP Add 135 µl **Assay Buffer** to the vial. Further dilute this predilution:

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

16. TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Assay Buffer**, in duplicate, to all standard wells
- Pipette 100 µl prediluted **sCD8 Standard** into the first wells and create standard dilutions ranging from 2000 to 31.25 U/ml by transferring 100 µl from well to well. Discard 100 µl from the last w.
- Add 100 µl **Assay Buffer**, in duplicate, to the blank wells
- Add 50 µl **Assay Buffer**, in duplicate, to the sample wells
- Add 50 µl **Sample**, in duplicate, to designated wells
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) on microplate shaker
- Prepare **Detection Antibody**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of diluted **Detection Antibody** to all wells
- Cover microwell strips and incubate 1 hours at room temperature (18° to 25°C) on microplate shaker
- Prepare **anti-rabbit-HRP**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of diluted **anti-rabbit-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 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank w.
- Incubate the microwell strips for 15 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 standard1 may result in incorrect sCD8 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual sCD8

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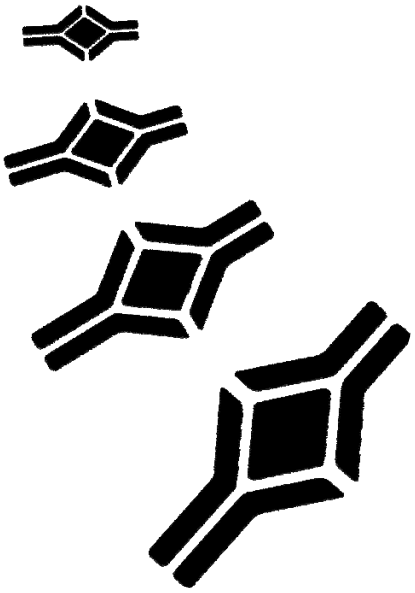

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