

Human sCD44std ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human sCD44std

Catalog Numbers BMS209-2 and BMS209-2TEN

Pub. No. MAN0016538 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Human sCD44std ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human sCD44std.

Summary

CD44 (Pgp-1; Ly-24; ECMR III; F10-44-2; H-CAM; HUTCH-I; In(Lu)-related p80; Hermes antigen; hyaluronan receptor) is a polymorphic glycoprotein with apparent molecular weights ranging from 85kDa to 250kDa. This cell membrane associated molecule has a cytoplasmic tail (mediates the interaction with the cytoskeleton), a short hydrophobic transmembrane region and an NH₂-terminal extracellular (binds to hyaluronate) domain.

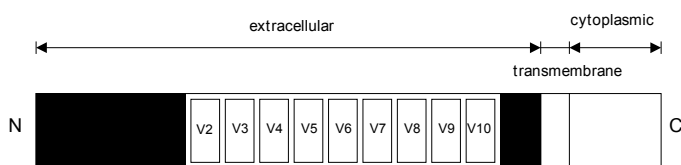
CD44 isoforms participate in a wide variety of cell-cell or cell-matrix interactions including lymphocyte homing, establishment of B- and T-cell immune responses, tumor metastases formation, and inflammation.

Three isoform categories of the CD44 molecule have been identified:

- An 80–90kDa isoform, the so-called standard form named CD44std, which is widely distributed on several hematopoietic and nonhemato-poietic cells including all subsets of leukocytes, monocytes, erythrocytes, many types of epithelium, mesenchymal elements like fibroblasts, smooth muscle cells, and glial cells of the central nervous system,
- A medium size category of 110–160kDa, which is weakly expressed on epithelial cells and highly expressed in some carcinomas, and
- A category that includes very large isoforms of 250kDa covalently modified by the addition of chondroitin sulfate.

These bigger isoforms of CD44 arise by alternative splicing of one or more *variant* exons (v2–v10) into the extracellular part of the 90kDa constant form molecule. Compared to the standard CD44, all larger isoforms are expressed in a much more restricted fashion, only in a few normal tissues or on the surface of certain tumor cells. Some splice variants of CD44 play important and distinct roles in tumor metastasis.

The Human sCD44std ELISA Kit detects all circulating CD44 isoforms comprising the standard protein sequences (black area).



CD44 protein: - standard protein sequences (black area)
- variant exons (open boxes numbered v2 - v10)

For literature update refer to our website.

For Research Use Only. Not for use in diagnostic procedures.

Principles of the test

An anti-human sCD44std coating antibody is adsorbed onto microwells.

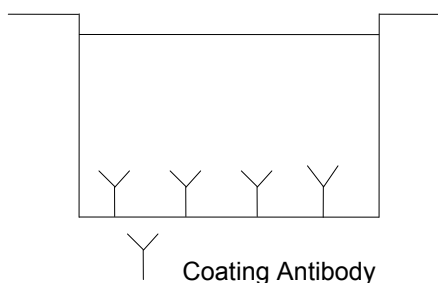


Fig. 1 Coated microwell.

Human sCD44std present in the sample or standard binds to antibodies adsorbed to the microwells. An HRP-conjugated anti-human sCD44std antibody is added, and binds to human sCD44std captured by the first antibody.

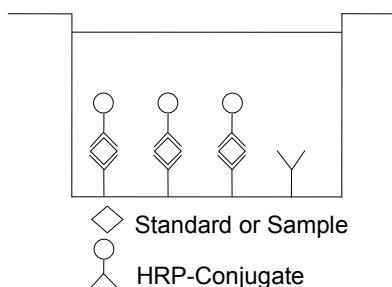


Fig. 2 First incubation.

Following incubation unbound HRP-conjugated anti-human sCD44std is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

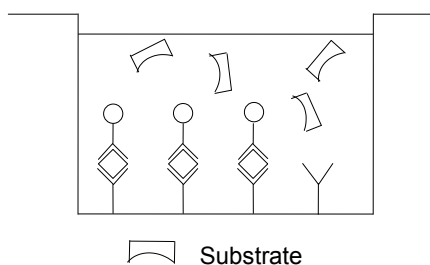


Fig. 3 Second incubation.

A colored product is formed in proportion to the amount of human sCD44std 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 sCD44std standard dilutions and human sCD44std concentration is determined.

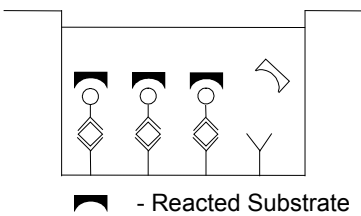


Fig. 4 Stop reaction.

Reagents provided

Reagents for human sCD44std ELISA BMS209-2 (96 tests)

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human sCD44std

2 vials HRP-Conjugate anti-human sCD44std monoclonal antibody

2 vials (230 μ L) human sCD44std Standard, 8 ng/mL

1 bottle (60 mL) Sample Diluent

1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 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 (15 mL) Stop Solution (1M phosphoric acid)

2 Adhesive Films

Reagents for human sCD44std ELISA BMS209-2TEN (10x96 tests)

10 aluminum pouches with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human sCD44std

10 vials HRP-Conjugate anti-human sCD44std monoclonal antibody

10 vials (230 μ L) human sCD44std Standard, 8 ng/mL

10 bottles (60 mL) Sample Diluent

1 vials (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)

3 bottles (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M phosphoric acid)

10 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). 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.

Sample collection and storage instructions

Cell culture supernatant, serum, plasma (EDTA, citrate, heparin), amniotic fluid, and urine 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. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human sCD44std. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (refer to “Sample stability” on page 6). 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 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
- 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and 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 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 pipet 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 samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that 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 samples 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.

Preparation of reagents

1. Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1x)

1. 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.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2°C to 25°C. The Wash Buffer (1x) is stable for 30 days.
4. Wash Buffer (1x) may also be prepared as needed according to the following table:

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

Assay buffer (1x)

1. 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.
2. Store at 2°C to 8°C. The Assay Buffer (1x) is stable for 30 days.
3. 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

HRP-Conjugate

Note: The HRP-Conjugate should be used within 30 minutes after dilution.

1. Dilute the HRP-Conjugate just prior to use by adding 490 μ L Assay Buffer (1x) to the tube containing the HRP-Conjugate concentrate. Mix the contents of the tube well.
2. Make a further 1:40 dilution with Assay Buffer (1x) in a clean plastic tube or reagent reservoir.
3. The second dilution (1:40) of the HRP-Conjugate may be prepared as needed according to the following table:

Number of Strips	Prediluted HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1-6	0.075	2.925
1-12	0.150	5.850

Human sCD44std standard

- Standard dilutions can be prepared directly on the microwell plate (see "Test protocol" on page 3) or alternatively in tubes (see "External standard dilution" on page 3).

External standard dilution

1. Label 6 tubes, one for each standard point: S1, S2, S3, S4, S5, S6.
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 μ L of Sample Diluent into each tube.
3. Pipette 225 μ L of undiluted standard (concentration = 8 ng/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 4 ng/mL). Pipette 225 μ L of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.

4. Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 5).

Sample Diluent serves as blank.

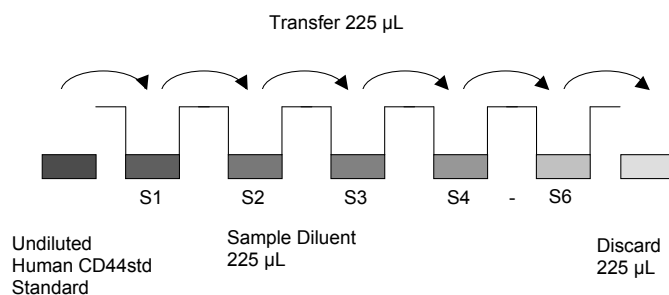


Fig. 5 Dilute standards - tubes.

Test protocol

Note: In case of incubation without shaking, the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

1. Predilute your serum, plasma, and urine samples before starting with the test procedure.

Dilute these samples 1:60 with Sample Diluent according to the following scheme:

10 μ L sample + 590 μ L Sample Diluent

2. 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°C to 8°C sealed tightly.
3. Wash the microwell strips twice with approximately 400 μ L 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.
4. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see "External standard dilution" on page 3):

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.

Add 100 μ L of Sample Diluent in duplicate to all standard wells. Pipet 100 μ L of undiluted standard (see "Human sCD44std standard" on page 3, concentration = 8.00 ng/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 4.00 ng/mL), and transfer 100 μ L to wells B1 and B2, respectively (see Figure 6). Take care not to scratch the inner surface of the microwells. Continue this procedure 4 times, creating two rows of human sCD44std standard dilutions ranging from 4.00 to 0.13 ng/mL. Discard 100 μ L of the contents from the last microwells (F1, F2) used.

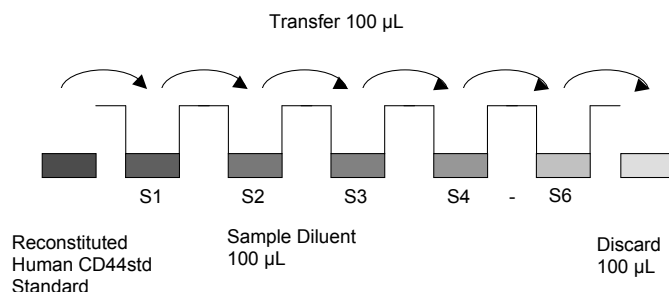


Fig. 6 Dilute standards - microwell plate.

In case of an external standard dilution (see “External standard dilution” on page 3), pipet 100 μ L of these standard dilutions (S1–S6) in the standard wells according to Table 1.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 4.00 ng/mL	Standard 1 4.00 ng/mL	Sample 2	Sample 2
B	Standard 2 2.00 ng/mL	Standard 2 2.00 ng/mL	Sample 3	Sample 3
C	Standard 3 1.00 ng/mL	Standard 3 1.00 ng/mL	Sample 4	Sample 4
D	Standard 4 0.50 ng/mL	Standard 4 0.50 ng/mL	Sample 5	Sample 5
E	Standard 5 0.25 ng/mL	Standard 5 0.25 ng/mL	Sample 6	Sample 6
F	Standard 6 0.13 ng/mL	Standard 6 0.13 ng/mL	Sample 7	Sample 7
G	Blank	Blank	Sample 8	Sample 8
H	Sample 1	Sample 1	Sample 9	Sample 9

- Add 100 μ L of Sample Diluent in duplicate to the blank wells.
- Add 80 μ L of Sample Diluent to the sample wells.
- Add 20 μ L of each sample in duplicate to the sample wells.
- Prepare HRP-Conjugate (see “HRP-Conjugate” on page 3).
- Add 50 μ L of HRP-Conjugate to all wells.
- Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours on a microplate shaker.
- Remove adhesive film and empty wells. Wash microwell strips 3 times according to step 3. Proceed immediately to the next step.
- Pipet 100 μ L of TMB Substrate Solution to all wells.
- Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for color 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 color. Alternatively the color 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 0.9–0.95.

- Stop the enzyme reaction by quickly pipetting 100 μ L 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°C to 8°C in the dark.
- 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 standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human sCD44std 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 sCD44std 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 sCD44std concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:300 (1:60 predilution, 1:5 dilution on the plate: 20 μ L sample + 80 μ L Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (\times 300).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human sCD44std levels. Such samples require further external predilution according to expected human sCD44std values with Sample Diluent in order to precisely quantitate the actual human sCD44std level.
- It is suggested that each testing facility establishes a control sample of known human sCD44std 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 7.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

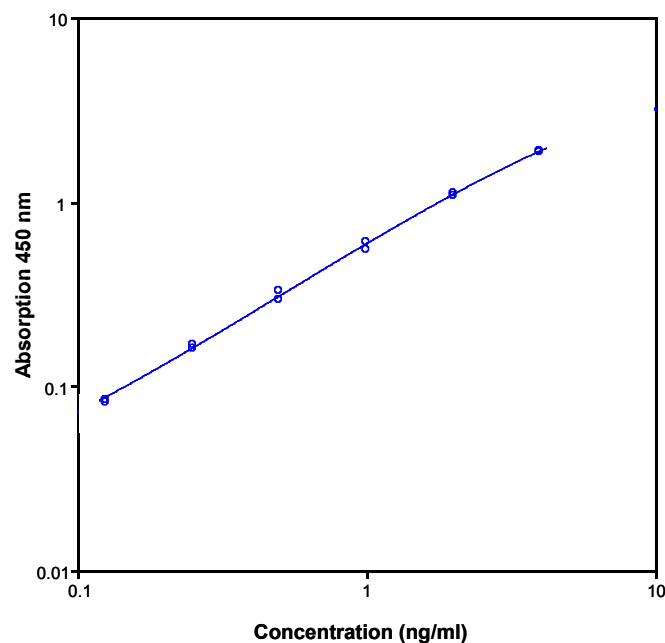


Fig. 7 Representative standard curve for Human sCD44std ELISA Kit . Human sCD44std was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the Human sCD44std ELISA Kit .

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human sCD44std concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	4.00	1.901 1.851	1.876	1.9
2	2.00	1.080 1.109	1.095	1.9
3	1.00	0.608 0.553	0.581	6.7
4	0.50	0.330 0.293	0.312	8.4
5	0.25	0.160 0.168	0.164	3.4
6	0.13	0.081 0.084	0.083	2.6
Blank	0.0	0.008 0.007	0.008	6.7

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 color intensity. Values measured are still valid.

Limitations

- Because 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 analyzed 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 sCD44std 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 0.02 ng/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 2 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human sCD44std. Two standard curves were run on each plate. Data below show the mean human sCD44std concentration and the coefficient of variation for

each sample. The calculated overall intra-assay coefficient of variation was 4.8%.

Table 3 The mean human sCD44std concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean concentration (ng/mL)	Coefficient of variation (%)
1	1	292	4.4
	2	343	3.8
2	1	332	5.9
	2	361	1.4
3	1	295	2.4
	2	291	8.3
4	1	318	9.6
	2	345	3.7
5	1	177	3.7
	2	173	1.1
6	1	437	4.1
	2	427	4.1
7	1	370	8.1
	2	370	3.3
8	1	297	11.3
	2	278	1.2

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 sCD44std. Two standard curves were run on each plate. Data below show the mean human sCD44std concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall inter-assay coefficient of variation was 4.1%.

Table 4 The mean human sCD44std concentration and the coefficient of variation of each sample.

Sample	Mean concentration (ng/mL)	Coefficient of variation (%)
1	318	11.3
2	297	6.9
3	293	1.1
4	332	5.8
5	175	1.4
6	432	1.5
7	370	0.1
8	288	4.5

Spike recovery

The spike recovery was evaluated by spiking four levels of human sCD44std into pooled normal human serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The amount of endogenous human sCD44std in unspiked serum was subtracted from the spike values. The recovery ranged from 76–101% with an overall mean recovery of 89%.

Dilution parallelism

Four serum samples with different levels of human sCD44std were analyzed at serial 2-fold dilutions with four replicates each. The recovery ranged from 91% to 99% with an overall recovery of 94%.

Sample	Dilution	Expected concentration (ng/mL)	Observed concentration (ng/mL)	Recovery of expected concentration (%)
1	1:300	–	347	–
	1:600	173	160	92
	1:1200	87	80	92
	1:2400	43	42	96
2	1:300	–	402	–
	1:600	201	186	92
	1:1200	100	94	94
	1:2400	50	46	91
3	1:300	–	292	–
	1:600	146	145	99
	1:1200	73	72	99
	1:2400	37	34	92
4	1:300	–	368	–
	1:600	184	173	94
	1:1200	92	89	96
	1:2400	46	43	94

Sample stability

Freeze-thaw stability

Aliquots of serum samples were stored at –20°C and thawed 5 times, and the human sCD44std levels determined. There was no significant loss of human sCD44std immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum samples were stored at –20°C, 2°C to 8°C, room temperature, and at 37°C, and the human sCD44std level determined after 24 hours. There was no significant loss of human sCD44std immunoreactivity detected during storage under above conditions.

Comparison of serum and plasma

Serum, as well as EDTA, citrate, and heparin plasma, from 22 individuals was obtained at the same time point. All these blood preparations were found suitable for human sCD44std determinations, although human sCD44std levels in citrate and EDTA plasma were slightly lower than serum levels. It is, therefore, highly recommended to ensure the uniformity of sample preparations.

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human CD44std positive serum. No cross-reactivity was detected, namely not with TNF α , TNF β , TNF-R, IFN α 2C, IFN γ , IL-8, Annexin, ELAM-1, L-selectin, sICAM-1, and HER-2.

Expected values

A panel of 22 sera samples from randomly selected apparently healthy donors (males and females) was tested for human sCD44std. The detected human sCD44std levels ranged between 251 and 925 ng/mL with a mean level of 443 ng/mL and a standard deviation of 125 ng/mL. The levels measured may vary with the sample collection used.

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

HRP-Conjugate

- Add 490 μ L Assay Buffer (1x) to the vial containing the HRP-Conjugate concentrate.
- Make a further 1:40 dilution in Assay Buffer (1x):

Number of Strips	Prediluted HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1–6	0.075	2.925
1–12	0.150	5.850

Test protocol summary

1. Predilute serum, plasma and urine samples with Sample Diluent 1:60.
2. Determine the number of microwell strips required.
3. Wash microwell strips twice with Wash Buffer.
4. Standard dilution on the microwell plate: Add 100 μ L Sample Diluent, in duplicate, to all standard wells. Pipette 100 μ L undiluted standard into the first wells and create standard dilutions by transferring 100 μ L from well to well. Discard 100 μ L from the last wells.
Alternatively, external standard dilution in tubes (see “External standard dilution” on page 3): Pipette 100 μ L of these standard dilutions in the microwell strips.
5. Add 100 μ L Sample Diluent, in duplicate, to the blank wells.
6. Add 80 μ L Sample Diluent to sample wells.
7. Add 20 μ L prediluted sample in duplicate, to designated sample wells.
8. Prepare the HRP-Conjugate.
9. Add 50 μ L HRP-Conjugate to all wells.
10. Cover microwell strips and incubate 3 hours at room temperature (18°C to 25°C).
11. Empty and wash microwell strips 3 times with Wash Buffer.
12. Add 100 μ L of TMB Substrate Solution to all wells.
13. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
14. Add 100 μ L Stop Solution to all wells.
15. Blank microwell reader and measure color intensity at 450 nm.

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- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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