

Human sCD44var (v5) ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human sCD44var (v5)

Catalog Numbers BMS220 and BMS220TEN

Pub. No. MAN0016575 **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 sCD44var (v5) ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human CD44var (v5).

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 which participates in a wide variety of cell-cell or cell-matrix interactions including lymphocyte homing, establishment of B- and T-cell immune responses, tumor metastasis formation and inflammation.

Three isoform categories of the CD44 molecule have been identified:

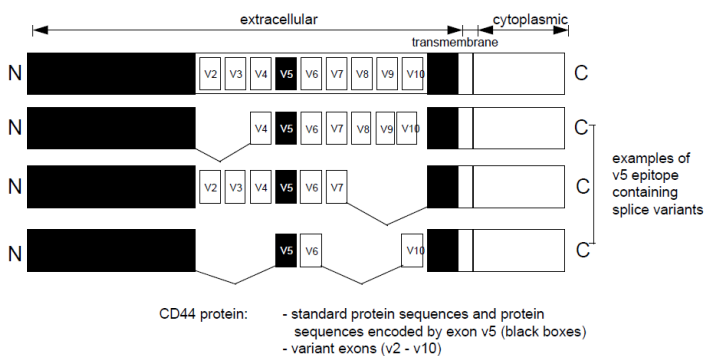
- a predominant 80-90 kDa category, the so-called standard form named CD44std,
- an intermediate size category of 110-160 kDa, and
- a category which includes very large isoforms of 250 kDa covalently modified by the addition of chondroitin sulfate.

This CD44-family of transmembrane receptor molecules is derived from a single gene located on chromosome 11. Alternative splicing of the mRNA gives rise to the different isoforms, containing inserts of varying sizes in the extracellular domain of the molecule (exons v2-v10). All CD44 isoforms are variably glycosylated. In contrast to standard CD44 (CD44std) which is almost ubiquitously expressed, the variety of CD44 isoforms (CD44var) have a much more restricted distribution, e.g., on keratinocytes (exons v3-v10), epithelial cells (exons v(-v10), activated lymphocytes and macrophages (exon v6).

A splice variant of CD44 (exons v4-v7) confers metastatic behavior in a rat carcinoma model; aberrant expression of splice variants has been detected on a variety of human tumor cell lines as well as primary and metastatic human tumors, including lymphomas, carcinomas (colon, thyroid, mamma, bladder), and gliomas.

Detection of abnormal regulation of CD44 splicing thus could be helpful in cancer diagnosis and disease evaluation.

The sCD44var (v5) ELISA detects all circulating CD44 isoforms comprising the CD44var (v5) sequences.



For literature update refer to our website.

Principles of the test

An anti-human CD44var (v5) coating antibody is adsorbed onto microwells.

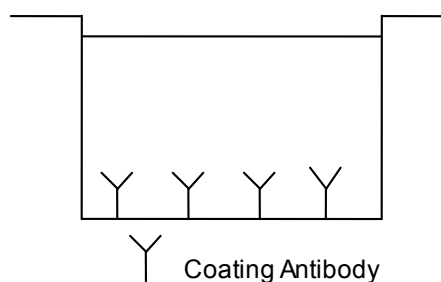


Fig. 1 Coated microwell

Human CD44var (v5) present in the sample or standard binds to antibodies adsorbed to the microwells. A HRP-conjugated anti-human CD44var (v5) antibody is added and binds to human CD44var (v5) captured by the first antibody.

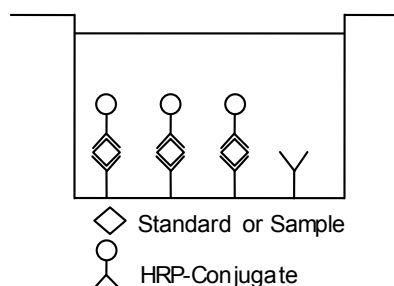


Fig. 2 First incubation

Following incubation unbound HRP-conjugated anti-human CD44var (v5) is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

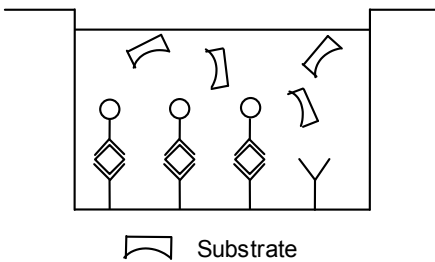


Fig. 3 Second incubation

A colored product is formed in proportion to the amount of human CD44var (v5) 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 CD44var (v5) standard dilutions and human CD44var (v5) concentration determined.

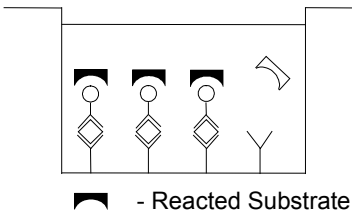


Fig. 4 Stop reaction

Reagents provided

Reagents for human CD44var (v5) ELISA BMS220 (96 tests)

- 1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human CD44var (v5)
- 2 vials HRP-Conjugate anti-human CD44var (v5) monoclonal antibody
- 2 vials human CD44var (v5) Standard lyophilized, 20 ng/mL upon reconstitution
- 1 bottle (50 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 CD44var (v5) ELISA BMS220TEN (10x96 tests)

- 10 aluminum pouches with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human CD44var (v5)
- 10 vials HRP-Conjugate anti-human CD44var (v5) monoclonal antibody
- 10 vials human CD44var (v5) Standard lyophilized, 20 ng/mL upon reconstitution
- 5 bottles (50 mL) Sample Diluent
- 1 vial (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 and 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.

Pay attention to a possible *Hook Effect* due to high sample concentrations (see “Calculation of results” on page 4).

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 CD44var (v5). If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (for sample stability 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 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for 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

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

Wash buffer (1x)

- Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water.
- Mix gently to avoid foaming.
- Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
- Wash Buffer (1x) may also be prepared as needed according to the following table:

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

Assay buffer (1x)

- Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
- Store at 2°C to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
- Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

HRP-Conjugate

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

- Dilute the HRP-Conjugate 1:20 just prior to use by adding 190 µL Assay Buffer (1x) to the tube containing the HRP-Conjugate concentrate. Mix the contents of the tube well.
- Make a further 1:100 dilution with Assay Buffer (1x) in a clean plastic tube.
- The second dilution (1:100) of the HRP-Conjugate may be prepared as needed according to the following table:

Number of Strips	Prediluted (1:20) HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Human CD44var (v5) standard

- Reconstitute human CD44var (v5) standard by addition of distilled water.
- Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 20 ng/mL).
- Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

The standard has to be used immediately after reconstitution and cannot be stored.

External standard dilution

- Label 6 tubes, one for each standard point: S1, S2, S3, S4, S5, S6.
- Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
- Pipette 225 µL of reconstituted standard (concentration = 20 ng/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 10 ng/mL).
- Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
- Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 5).

Sample Diluent serves as blank.

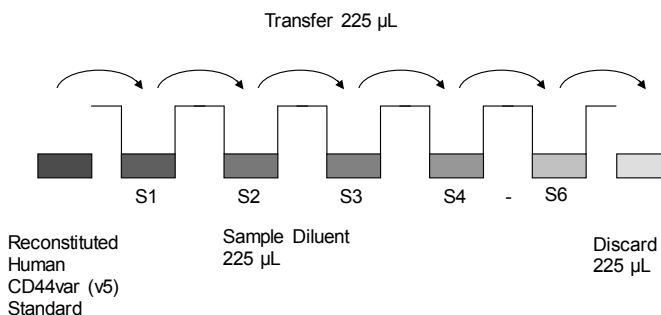


Fig. 5 Dilute standards - tubes

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 samples before starting with the test procedure. Dilute serum, plasma, and urine samples 1:6 with Sample Diluent according to the following scheme: 20 μ L sample + 100 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.

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.

4. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see “External standard dilution” on page 3):

Add 100 μ L of Sample Diluent in duplicate to all standard wells. Pipette 100 μ L of prepared standard (see “Human CD44var (v5) standard” on page 3, concentration = 20.00 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 = 10.00 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 CD44var (v5) standard dilutions ranging from 10.00 to 0.31 ng/mL. Discard 100 μ L of the contents from the last microwells (F1, F2) used.

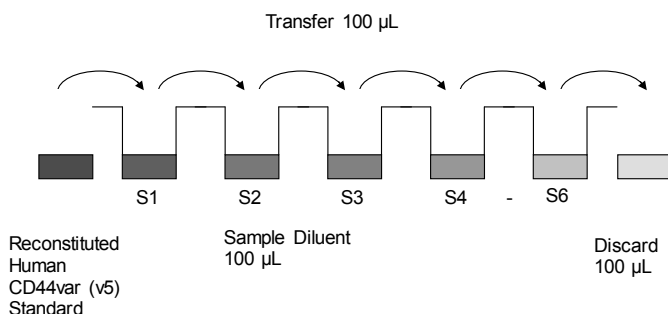


Fig. 6 Dilute standards - microwell plate.

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

	1	2	3	4
A	Standard 1 10.00 ng/mL	Standard 1 10.00 ng/mL	Sample 2	Sample 2
B	Standard 2 5.00 ng/mL	Standard 2 5.00 ng/mL	Sample 3	Sample 3
C	Standard 3 2.50 ng/mL	Standard 3 2.50 ng/mL	Sample 4	Sample 4
D	Standard 4 1.25 ng/mL	Standard 4 1.25 ng/mL	Sample 5	Sample 5
E	Standard 5 0.63 ng/mL	Standard 5 0.63 ng/mL	Sample 6	Sample 6
F	Standard 6 0.31 ng/mL	Standard 6 0.31 ng/mL	Sample 7	Sample 7
G	Blank	Blank	Sample 8	Sample 8
H	Sample 1	Sample 1	Sample 9	Sample 9

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

5. Add 100 μ L of Sample Diluent in duplicate to the blank wells.
6. Add 80 μ L of Sample Diluent to the sample wells.
7. Add 20 μ L of each prediluted sample in duplicate to the sample wells.
8. Prepare HRP-Conjugate (see “HRP-Conjugate” on page 3).
9. Add 50 μ L of HRP-Conjugate to all wells.
10. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours on a microplate shaker.
11. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 3. of the test protocol. Proceed immediately to the next step.
12. Pipette 100 μ L of TMB Substrate Solution to all wells.
13. 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 point of this protocol) 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.

14. 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.
15. 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 CD44var (v5) 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 CD44var (v5) 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 CD44var (v5) concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:30 (1:6 external 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 ($\times 30$).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human CD44var (v5) levels (Hook Effect). Such samples require further external predilution according to expected human CD44var (v5) values with Sample Diluent in order to precisely quantitate the actual human CD44var (v5) level.

- It is suggested that each testing facility establishes a control sample of known human CD44var (v5) 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.

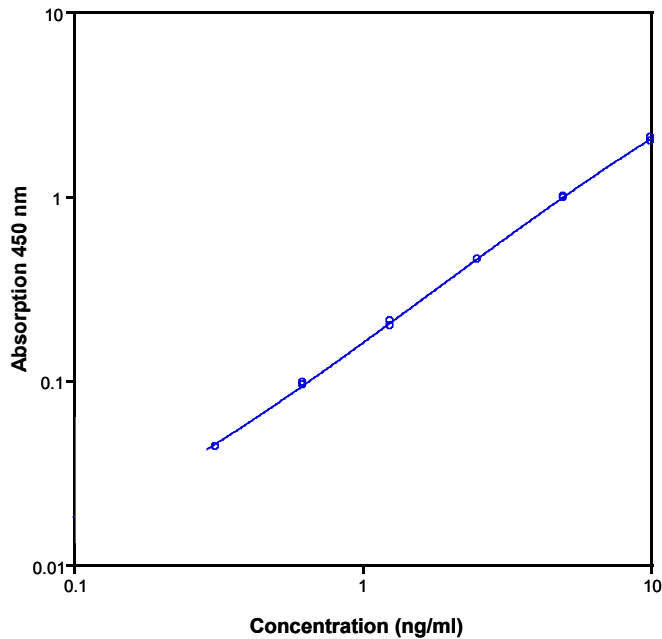


Fig. 7 Representative standard curve for human CD44var (v5) ELISA. Human CD44var (v5) was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the human CD44var (v5) ELISA.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human CD44var (v5) Concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	10.00	1.997 2.071	2.034	2.5
2	5.00	0.997 0.981	0.989	1.0
3	2.50	0.451 0.454	0.453	0.6
4	1.25	0.210 0.198	0.204	3.7
5	0.63	0.094 0.097	0.095	1.8
6	0.31	0.044 0.044	0.044	0.0
Blank	0.0	0.022 0.026	0.024	8.3

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

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be 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 CD44var (v5) 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 0.14 ng/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human CD44var (v5). Two standard curves were run on each plate. Data below show the mean human CD44var (v5) concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 3.6%.

Table 3 The mean human CD44var (v5) concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean human CD44var (v5) concentration (ng/mL)	Coefficient of variation (%)
1	1	66	3.0
	2	65	3.2
	3	65	4.3
2	1	32	2.6
	2	34	4.4
	3	33	1.3
3	1	194	2.5
	2	208	2.0
	3	244	1.6
4	1	146	4.5
	2	166	1.6
	3	160	2.5
5	1	119	3.6
	2	136	1.2
	3	126	2.6
6	1	48	6.4
	2	55	6.2
	3	51	3.9
7	1	52	9.9
	2	59	4.1
	3	56	2.8
8	1	42	7.3
	2	59	2.3
	3	55	3.0

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 CD44var (v5). Two standard curves were run on each plate. Data below show the mean human CD44var (v5) concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 5.8%.

Table 4 The mean human CD44var (v5) concentration and the coefficient of variation of each sample.

Sample	Mean human CD44var (v5) concentration (ng/mL)	Coefficient of variation (%)
1	66	0.6
2	33	1.7
3	215	9.8
4	157	5.3
5	127	5.3
6	51	5.1
7	56	4.8
8	52	13.8

Spike recovery

The spike recovery was evaluated by spiking 4 levels of human CD44var (v5) into different pooled normal serum samples. Recoveries were determined in 2 independent experiments with 4 replicates each. The amount of endogenous human CD44var (v5) in unspiked serum was subtracted from the spike values. The recovery ranged from 84–100% with an overall mean recovery of 92%.

Dilution parallelism

Four serum samples with different levels of human CD44var (v5) were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged from 98% to 129% with an overall recovery of 110%.

Sample	Dilution	Expected human CD44var (v5) concentration (ng/mL)	Observed human CD44var (v5) concentration (ng/mL)	Recovery of expected human CD44var (v5) concentration (%)
1	1:30	–	67	–
	1:60	34	41	121
	1:120	20	21	104
	1:240	10	10	98
2	1:30	–	200	–
	1:60	100	123	123
	1:120	62	72	117
	1:240	36	36	101
3	1:30	–	170	–
	1:60	85	106	125
	1:120	53	60	114
	1:240	30	32	107
4	1:30	–	140	–
	1:60	70	90	129
	1:120	45	53	117
	1:240	26	26	101

Sample stability

Freeze-Thaw stability

Aliquots of serum samples were stored at -20°C and thawed 5 times, and the human CD44var (v5) levels determined. There was no significant loss of human CD44var (v5) 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 CD44var (v5) level determined after 24 hours. There was no significant loss of human CD44var (v5) immunoreactivity detected during storage under above conditions.

Comparison of serum and plasma

Serum, as well as EDTA, citrate and heparin plasma samples from 22 individuals were obtained at the same time point. All these blood preparations were found suitable for human CD44var (v5) determinations, although human CD44var (v5) levels in citrate, EDTA and heparin plasma were slightly lower than serum levels. It is, therefore, highly recommended to assure the uniformity of sample preparations.

Specificity

The assay recognizes both natural and recombinant forms of the CD44var (v5) molecule. The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human CD44var (v5) positive serum. No cross-reactivity was detected, namely not with human CD44 polypeptides lacking the protein sequence encoded by exon 5.

Expected values

A panel of 22 serum samples from randomly selected apparently healthy donors (males and females) was tested for human CD44var (v5). The detected human CD44var (v5) levels ranged between 6 and 55 ng/mL with a mean level of 35 ng/mL and a standard deviation of 13 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

Make a 1:20 predilution of the HRP-Conjugate in 190 µL Assay Buffer (1x). Make a further 1:100 dilution in Assay Buffer (1x):

Number of Strips	Prediluted (1:20) HRP-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Human CD44var (v5) standard

Reconstitute lyophilized human CD44var (v5) standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

Note: If instructions in this protocol have been followed, samples have been diluted 1:30 (1:6 external predilution, 1:5 dilution on the plate: 20 µL sample + 80 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 30).

1. Predilute serum, plasma, and urine samples with Sample Diluent 1:6.
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 µL Sample Diluent, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

Alternatively, external standard dilution in tubes (see “External standard dilution” on page 3): Pipette 100 µL of these standard dilutions in the microwell strips.
5. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
6. Add 80 µL Sample Diluent to sample wells.
7. Add prediluted 20 µL sample in duplicate to designated sample wells.
8. Prepare HRP-Conjugate.
9. Add 50 µL 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 µL 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 µL Stop Solution to all wells.
15. Blank microwell reader and measure color intensity at 450 nm.

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.



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

The information in this guide is subject to change without notice.

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