ELISA PRODUCT INFORMATION & MANUAL

Human ICAM-2/CD102 ELISA Kit (Colorimetric)

NBP1-91181

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Human ICAM-2/CD102 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human ICAM-2/CD102

Catalog Number NBP1-91181

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 Technical Support.

Product description

The Human ICAM-2/CD102 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human Intercellular Adhesion Molecule-2 (ICAM-2).

Summary

Intercellular Adhesion Molecule-2 (ICAM-2, CD102) is a 60 kDa surface glycoprotein that promotes adhesion between immune cells and the vascular endothelium. ICAM-2 is a member of the Ig-superfamily with two immunoglobulin-like extracellular domains. ICAM-2 is much more closely related to the two N-terminal domains of ICAM-1 (34%identity) than either ICAM-1 or ICAM-2 is to other members of the Ig-superfamily, demonstrating the existence of a subfamily of Ig-like ligands that bind the same integrin receptor. ICAM-2 is broadly distributed on hematopoietic cells. On resting lymphocytes, the ICAM-2 expression is several-fold higher than that seen with ICAM-1, while monocytes express equivalent levels of ICAM-1 and ICAM-2. In contrast to ICAM-1, neutrophils stain negative for ICAM-2. Just little or no ICAM-2 staining can be demonstrated on other cell lines, the sole exception being (i) resting vascular endothelial cells, which possess high levels of ICAM-2, and (ii) small clusters of cells in lymphoid tissue germinal centres.

ICAM-2 expression is unaffected by a variety of inflammatory cytokines, while ICAM-1 is upregulated upon stimulation. This points toward the importance of ICAM-2 in the unstimulated resting state, before ICAM-1 expression is increased.

The integrin LFA-1 is the receptor for ICAM-2 and ICAM-1 as well. The functional characteristics of ICAM-2 as LFA-1 ligand can be summarized as follows:

ICAM-2 dominates over ICAM-1 on resting endothelial cells and seems to be involved in the recirculation of LFA-1 positive lymphocytes, e.g., facilitating T-memory cell recirculation. As resting T-cells express little or no ICAM-1, ICAM-2 may also be important in initial T-cell adhesion with antigen-presenting cells that bear LFA-1. Also the lysis of certain target cells appears to occur in an ICAM-1 independent manner, possibly regulated by ICAM-2.

Principles of the test

An anti-human ICAM-2/CD102 coating antibody is adsorbed onto microwells.

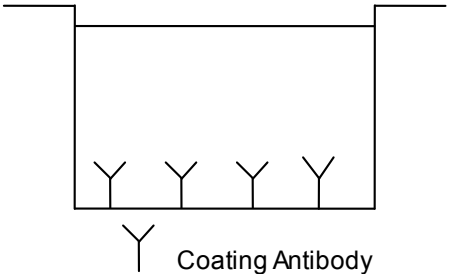

Fig. 1 Coated microwell

Human ICAM-2/CD102 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human ICAM-2/CD102 antibody is added and binds to human ICAM-2/CD102 captured by the irst antibody.

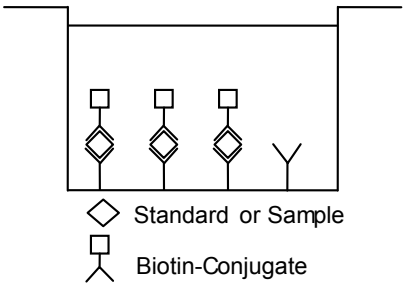

Fig. 2 First incubation

Following incubation unbound biotin-conjugated anti-human ICAM-2/CD102 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IICAM-2/CD102 antibody.

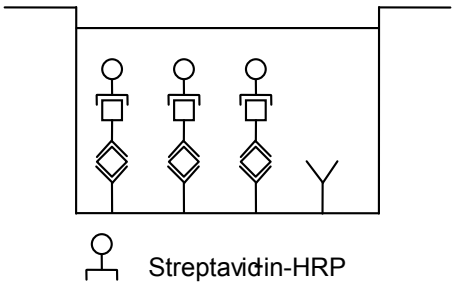

Fig. 3 Second incubation

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

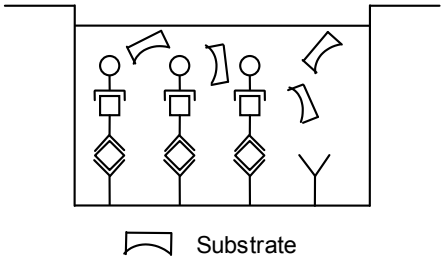

Fig. 4 Third incubation

A colored product is formed in proportion to the amount of human ICAM-2/CD102 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 ICAM-2/CD102 standard dilutions and human ICAM-2/CD102 sample concentration determined.

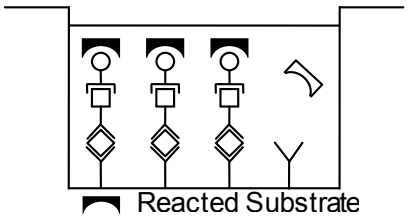

Fig. 5 Stop reaction

Reagents provided

1 aluminum pouch with a Microwell Plate (12 strips with 8 wells each) coated with monoclonal antibody to human ICAM-2/CD102

1 vial (0.4 mL) Biotin-Conjugate anti-human ICAM-2/CD102 monoclonal antibody

2 vials (5 µL) Streptavidin-HRP-Concentrate

2 vials human ICAM-2/CD102 Standard lyophilized, 24.00 U/mL upon reconstitution

1 bottle (23 mL) Streptavidin-HRP Diluent

1 bottle (25 mL) Standard Buffer Diluent (10x)

1 bottle (7.5 mL) Biotin Conjugate Diluent

1 bottle (10 mL) Wash Buffer Concentrate 200x

1 bottle (11 mL) Substrate Solution (tetramethyl-benzidine and buffered hydrogen peroxide)

1 vial (11 mL) Stop Solution (1N H_2 SO_4)

4 Adhesive Films

Storage instructions – ELISA kit

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.

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, urine, whole blood, 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 ICAM-2/CD102. If samples are to be run within 24 hours, they may be stored at 2–8°C (refer to "Sample stability" on page 5). 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

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 (10 mL) of the Wash Buffer Concentrate (200x) into a clean 2000 mL graduated cylinder. Bring to final volume of 2000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
3. 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	2.5	497.5
1 - 12	5	955

Standard buffer diluent (1x)

1. Pour the entire contents (25 mL) of the concentrated Standard Buffer Diluent (10x) into a clean 250 mL graduated cylinder. Bring to final volume of 250 mL with distilled water. Mix gently to avoid foaming.
2. Standard Buffer Diluent (1x) may also be prepared as needed according to the following table:

Number of Strips	Standard Buffer Diluent (10x) (mL)	Distilled Water (mL)
1 - 6	5.0	45
1 - 12	10.0	90.0

Biotin-Conjugate

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

Make a dilution (1:27.5) of the concentrated Biotin-Conjugate solution with Biotin-Conjugate Diluent in a clean plastic tube as needed according to the following table:

Number of Strips	Concentrated Biotin-Conjugate (mL)	Biotin Conjugate Diluent (mL)
1 - 6	0.11	2.89
1 - 12	0.22	5.78

Streptavidin-HRP

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

1. Dilute the Streptavidin-HRP just prior to use by adding 500 µL Streptavidin-HRP Diluent to the tube containing the Streptavidin-HRP concentrate (5 µL). Mix the contents of the tube well.
2. Make a further dilution with Streptavidin-HRP Diluent in a clean plastic tube or reagent reservoir according to the following table:

Number of Strips	Prediluted Streptavidin-HRP (mL)	Streptavidin-HRP Diluent (mL)
1 - 6	0.075	4.925
1 - 12	0.150	9.850

Human ICAM-2/CD102 standard

1. Reconstitute human ICAM-2/CD102 standard by addition of Standard Buffer Diluent (1x). Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilisation (concentration of reconstituted standard = 24.00 U/mL). Allow the reconstituted standard to sit for 10-30 minutes. Mix well prior to making dilutions.
2. The standard has to be used immediately after reconstitution and cannot be stored.

External standard dilution

1. Label 5 tubes, one for each standard point: S2, S3, S4, S5, S6.
2. Then prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Standard Buffer Diluent (1x) into tubes S2 – S6.
3. Pipette 225 µL of reconstituted standard (serves as the highest standard S1, concentration of standard 1 = 24.00 U/mL) into the first tube, labeled S2, and mix (concentration of standard 2 = 12.00 U/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S3, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 3 more times thus creating the points of the standard curve (see Figure 6).

   Standard Buffer Diluent (1x) serves as blank.

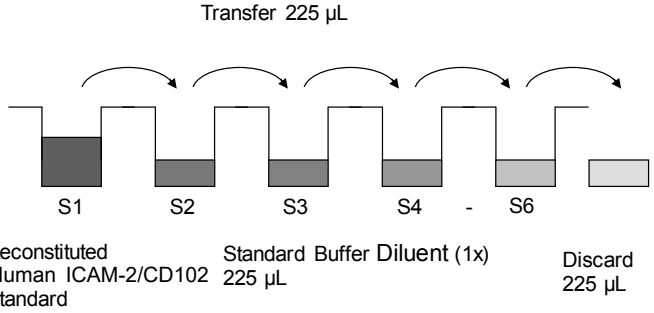

Fig. 6 Dilute standards - tubes

Test protocol

1. Predilute your samples before starting with the test procedure. Dilute serum and plasma samples 1:20 with Standard Buffer Diluent (1x) according to the following scheme:

   15 µL sample + 285 µL Standard Buffer Diluent (1x)
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°-8°C sealed tightly.
3. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes - see "External standard dilution" on page 3.):
   Add 100 µL of Standard Buffer Diluent (1x) in duplicate to standard wells B1/2-F1/2, leaving A1/A2 empty. Pipette 200 µL of prepared standard (see "Human ICAM-2/CD102 standard" on page 3) in duplicate into well A1 and A2 (see Table 1) (concentration of standard 1, S1 = 24.00 U/mL), and transfer 100 µL to wells B1 and B2, respectively. Mix the contents of wells B1 and B2 by repeated aspiration and ejection, and transfer 100 µL to wells C1 and C2, respectively. (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 3 times, creating two rows of human ICAM-2/CD102 standard dilutions ranging from 24.00 to 0.75 U/mL. Discard 100 µL of the contents from the last microwells (F1, F2) used.

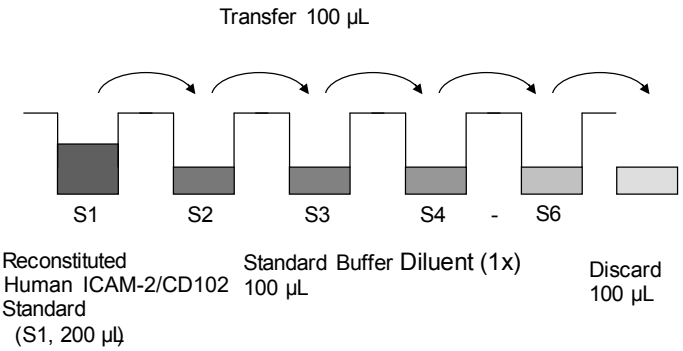

Fig. 7 Dilute standards - microwell plate

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

	1	2	3	4
A	Standard 1 24.00 U/mL	Standard 1 24.00 U/mL	Sample 2	Sample 2
B	Standard 2 12.00 U/mL	Standard 2 12.00 U/mL	Sample 3	Sample 3
C	Standard 3 6.00 U/mL	Standard 3 6.00 U/mL	Sample 4	Sample 4
D	Standard 4 3.00 U/mL	Standard 4 3.00 U/mL	Sample 5	Sample 5
E	Standard 5 1.50 U/mL	Standard 5 1.50 U/mL	Sample 6	Sample 6
F	Standard 6 0.75 U/mL	Standard 6 0.75 U/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 µL of these standard dilutions (S1 – S6) in the standard wells according to Table 1.

4. Add 100 µL of Standard Buffer Diluent (1x) in duplicate to the blank wells.
5. Add 100 µL of each prediluted sample in duplicate to the sample wells.
6. Prepare Biotin-Conjugate (see "Streptavidin-HRP" on page 3).
7. Add 50 µL of prepared Biotin-Conjugate to all wells.
8. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 1 hour, if available on a microplate shaker.
9. Prepare Streptavidin-HRP (refer to "Streptavidin-HRP" on page 3).
10. Remove adhesive film and empty wells. Wash the microwell strips 3 times 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. 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.
11. Add 100 µL of prepared Streptavidin-HRP to all wells, including the blank wells.
12. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 30 minutes, if available on a microplate shaker.
13. Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 10. of the test protocol. Proceed immediately to the next step.
14. Pipette 100 µL of TMB Substrate Solution to all wells.
15. Incubate the microwell strips at room temperature (18° to 25°C) for about 15 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.
16. Stop the enzyme reaction by quickly pipetting 100 µL 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 - 8°C in the dark.
17. 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.

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

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human ICAM-2 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 ICAM-2/CD102 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 ICAM-2/CD102 concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:20 (15 µL sample + 285 µL Standard Buffer Diluent (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 20).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human ICAM-2/CD102 levels. Such samples require further external predilution according to expected human ICAM-2/CD102 values with Standard Buffer Diluent (1x) in order to precisely quantitate the actual human ICAM-2/CD102 level.
- It is suggested that each testing facility establishes a control sample of known human ICAM-2/CD102 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 8. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

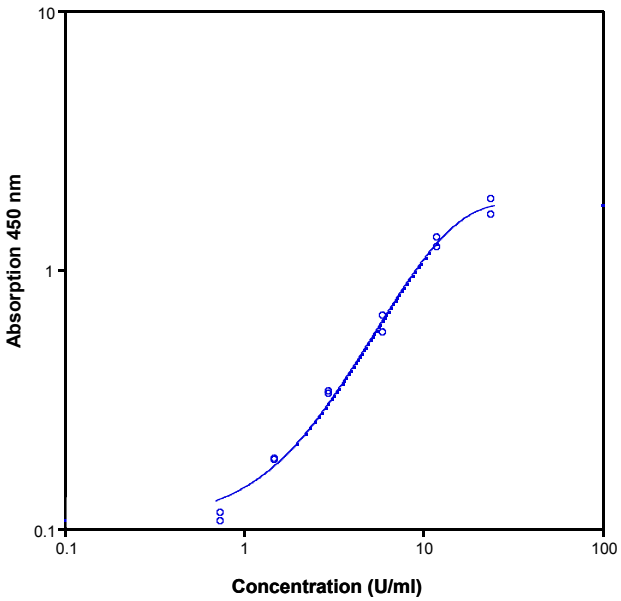

Fig. 8 Representative standard curve for human ICAM-2/CD102 ELISA. Human ICAM-2/CD102was diluted in serial 2-fold steps in Standard Buffer Diluent (1x). Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Table 2 Typical data using the human ICAM-2/CD102 ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human ICAM-2/ CD102 Concentration (U/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	24.00	1.627 1.869	1.748	6.9
2	12.00	1.220 1.328	1.274	4.3
3	6.00	0.569 0.665	0.617	7.8
4	3.00	0.331 0.339	0.335	1.2
5	1.50	0.183 0.186	0.184	0.7
6	0.75	0.115 0.107	0.111	3.6
Blank	0.00	0.032 0.032	0.032	0.0

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 ICAM-2/CD102 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 0.30 U/mL (mean of 20 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 10 independent experiments. Each assay was carried out with 6 replicates of 2 serum samples containing different concentrations of human ICAM-2/CD102.

2 standard curves were run on each plate. The calculated overall intra-assay coefficient of variation was 6.7%.

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 3 replicates of 2 serum samples containing different concentrations of human ICAM-2/CD102 2 standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was 7.0%.

Sample stability

Freeze-Thaw stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human ICAM-2/CD102 levels determined. There was no signi icant loss of human ICAM-2/CD102 immunoreactivity detected by freezing and thawing.

Storage stability

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

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into ICAM-2/CD102 positive serum. No cross-reactivity was detected, notably not with ICAM-1 and ICAM-3.

Expected values

A panel of 80 sera samples from randomly selected apparently healthy donors (males and females) was tested for human ICAM-2. The detected human ICAM-2/CD102 levels ranged between 44 and 405 U/mL with a mean level of 220 U/mL and a standard deviation of 74 U/mL.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 200x (10 mL) to 2000 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	497.5
1 - 12	5	955

Standard buffer diluent (1x)

Add Standard Buffer Diluent (10x) (25 mL) to 225 mL distilled water.

Number of Strips	Standard Buffer Diluent (10x) (mL)	Distilled Water (mL)
1 - 6	5.0	45
1 - 12	10.0	90.0

Biotin-Conjugate

Make a 1:27.5 dilution of concentrated Biotin-Conjugate in Biotin-Conjugate Diluent:

Number of Strips	Concentrated Biotin-Conjugate (mL)	Biotin-Conjugate Diluent (mL)
1 - 6	0.11	2.89
1 - 12	0.22	5.78

Streptavidin-HRP

Dilute 5 µL Streptavidin-HRP Concentrate in 500 µL Streptavidin-HRP Diluent. Make a further dilution according to the following table:

Number of Strips	Prediluted Streptavidin-HRP (µL)	Streptavidin-HRP Diluent (mL)
1 - 6	75	5
1 - 12	150	10

Human ICAM-2/CD102 standard

Reconstitute lyophilized human ICAM-2/CD102standard with Standard Buffer Diluent (1x). (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

1. Predilute sample 1:20 with Standard Buffer Diluent (1x).
2. Determine the number of microwell strips required.
3. Standard dilution on the microwell plate: Add 100 µL Standard Buffer Diluent (1x), in duplicate, to all standard wells leaving the first wells empty. Pipette 200 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.
4. Add 100 µL Standard Buffer Diluent (1x) in duplicate, to the blank wells.
5. Add 100 µL sample in duplicate, to designated sample wells.
6. Prepare Biotin-Conjugate.
7. Add 50 µL prepared Biotin-Conjugate to all wells.
8. Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
9. Prepare Streptavidin-HRP.
10. Empty and wash microwell strips 3 times with Wash Buffer.
11. Add 100 µL prepared Streptavidin-HRP to all wells.
12. Cover microwell strips and incubate 30 minutes at room temperature (18° to 25°C).
13. Empty and wash microwell strips 3 times with Wash Buffer.
14. Add 100 µL of TMB Substrate Solution to all wells.
15. Incubate the microwell strips for about 15 minutes at room temperature (18° to 25°C).
16. Add 100 µL Stop Solution to all wells.
17. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions in this protocol have been followed, samples have been diluted 1:20 (15 µL sample + 285 µL Standard Buffer Diluent (1x)) and the concentration read from the standard curve must be multiplied by the dilution factor (x 20).

Customer and technical support

Visit **https://www.novusbio.com/support** for service and support information.

Limited product warranty