Human ICAM-1
ELISA Kit

Cat. No. ECM335

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Background Information

Intercellular Adhesion Molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family [1] and functions as a ligand for integrin αLβ2 or Lymphocyte Function-Associated Antigen-1 (LFA-1), a member of the leukocyte integrin family [2] which mediates lymphocyte adhesion.

ICAM-1 is a single-chain glycoprotein with a polypeptide core of 55kDa expressed on non-hematopoietic cells of many lineages including vascular endothelial cells, thymic epithelial cells, other epithelial cells and fibroblasts, and on hematopoietic cells such as tissue macrophages, mitogen-stimulated T-lymphoblasts, germinal center B-cells and the dendritic cells from tonsils, lymph nodes and Peyer's patches. ICAM-1 is inducible on fibroblasts and endothelial cells by the inflammatory mediators IL-1, TNF and IFN-gamma. Its presence correlates with infiltration of lymphocytes into inflammatory lesions [3-5]. ICAM-1 seems to be the initial marker of inflammatory reactions and is expressed prior to and to a greater extent than HLA-DR.

The role of ICAM-1 as a disease marker has been demonstrated for a number of different pathologies, including allergic rhinitis, allergic contact dermatitis [6], gastrointestinal and bladder cancer, lymphoproliferative disorders [7,8], melanoma [9-11], HIV-1 infection [12], malaria, tissue or organ transplant rejection [13,14], diabetes mellitus [15], glomerulonephritis, asthma [16], rheumatoid arthritis [17] and psoriasis [18].

Test Principle

An anti-ICAM-1 monoclonal antibody is adsorbed onto microwells.

Soluble ICAM-1 present in a sample or standard then binds to antibodies adsorbed to the microwells. A second, HRP-conjugated monoclonal anti-ICAM-1 antibody is added and binds to ICAM-1 captured by the first antibody.

Unbound enzyme-conjugated anti-ICAM-1 is removed with a wash step and HRP substrate solution is added to the wells.

The pair of monoclonal antibodies used in this ELISA detect the soluble form of ICAM-1 present in serum, plasma, urine, and other biological fluids.

An amount of colored product is formed, proportional to the amount of soluble ICAM-1 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 five ICAM-1 standard dilutions and the ICAM-1 sample concentration is determined.

Application

The ICAM-1 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of soluble Intercellular Adhesion Molecule-1 in cell culture supernatants, serum, plasma, or other biological fluids. **The ICAM-1 ELISA is for research use only. Not for use in diagnostic procedures.**

Analytical Sensitivity and Detection Limits

Sensitivity:	2.17 ng/mL
Range of Detection:	6.25 - 100 ng/mL
Intra-Assay Variation:	4.1%
Inter-Assay Variation:	7.7%
Recovery:	98.6% average
Assay Time	75 minutes
Crossreactivity:	No interference from soluble TNF-R (60 / 80 kDa), IL-8/NAP-1, TNF-α, TNF-β, IFN-γ, IFN-α2C, IFN-ω, IL-6, IL-2R, E-selectin-1 or L-selectin-1.

Kit Components

- 1 aluminum pouch with Microwell plate coated with Monoclonal Antibody (murine) to human ICAM-1
- 1 vial (0.1 mL) HRP-Conjugated Anti-ICAM-1 Monoclonal (murine) Antibody
- 2 vials (0.5 mL each) 100 ng/mL soluble ICAM-1 Standard
- 1 vial Control (**high**), lyophilized
- 1 vial Control (**low**), lyophilized
- 1 bottle (50 mL) Wash Buffer Concentrate 20X (PBS with 1% Tween 20)
- 1 vial (5 mL) Assay Buffer Concentrate 20X (PBS with 2% Tween 20 and 10% BSA)
- 1 bottle (12 mL) Sample Diluent (buffered protein matrix)
- 1 vial (15 mL) Substrate Solution
- 1 vial (15 mL) Stop Solution (1M Phosphoric Acid)
- 1 Microwell Strip Holder
- 2 Adhesive Plate Covers

Materials Not Supplied

- 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 wavelength)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

Precautions

- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix / 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.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- 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 dedicated reagent trays for dispensing 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.

Sample Collection

Cell culture supernatants, human serum, EDTA-treated or heparinized plasma or other biological samples will be suitable for use in the assay. Remove serum or plasma 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.

Samples should be frozen at -20°C to avoid loss of bioactive ICAM-1. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

Sample Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2° to 8°C, room temperature and at 37°C, and the ICAM-1level determined after 24 hours. There was no significant loss of ICAM-1 immunoreactivity during storage.

Aliquots of serum samples (unspiked or spiked) were stored frozen at -20°C, thawed repeatedly, and ICAM-1 levels determined. There was no significant loss of ICAM-1 by 5 cycles of freezing and thawing.

Preparation of Reagents

All reagents with the exception of HRP-Conjugate (reagent C) should be prepared in advance before starting the test procedure.

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 1000 mL graduated cylinder. Bring final volume to 1000 mL with distilled or deionized water. Mix gently to avoid foaming. pH of final solution should be 7.4.

Transfer to a clean wash bottle and store between 2° and 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 HRP-Conjugate

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

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

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

D. Stop Solution (1M Phosphoric Acid)

Stop Solution is provided ready to use. Rubber or disposable gloves and protective glasses should be worn while handling Phosphoric Acid.

E. Preparation of Controls

Reconstitute the positive controls by adding 200 µL distilled water. Mix gently to ensure complete and homogeneous solublization. Treat the controls like a sample. For control range, please refer to the vial label. Store reconstituted control aliquots at -20ºC and avoid multiple freeze/thaw cycles.

Storage of Kit Components

Store the kit reagents, with the exception of the control serum, between 2° to 8°C. Store the lyophilized control at -20°C. Immediately after use, remaining reagents should be returned to 2° to 8°C with the exception of reconstituted control (-20ºC). Expiration date of the kit is stated on outside label.

Assay Instructions

a. Mix all reagents thoroughly before use. Avoid 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 should be assayed in duplicate. Remove sufficient Microwell Strips coated with Monoclonal Antibody to human ICAM-1 from their aluminum pouches prior to use. Load them into the 96 microwell strip holder making sure to place the first microwell strip into row 1.

c. Wash the microwell strips twice with approximately 300 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

   After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µL of Sample Diluent in duplicate to all standard wells, leaving the first wells (A1 and A2) empty. Prepare standard dilutions by pipetting 200 µL of soluble ICAM-1 Standard**,** in duplicate, into wells A1 and A2. 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. Take care not to scratch the inner surface of the microwells. Repeat this procedure twice, creating two rows of ICAM-1 standard dilutions ranging from 100 to 6.25 ng/mL. Discard 100 µL of the contents from the last microwells (E1, E2) used.

e. Add 100 µL of Sample Diluent in duplicate to the blank wells.

f. Add 90 µL of Sample Diluent to all wells designated for samples.

g. Add 10 µL of each Sample, in duplicate, to the designated wells and mix.

h. Prepare HRP-Conjugate. (Refer to preparation of reagents)

i. Add 50 µL of diluted HRP-Conjugate to all wells, including the blank wells.

j. Cover with a Plate Cover and incubate at room temperature (18° to 25°C) for 1 hour, preferably on a rotator set at 100 rpm.

k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c of the test protocol.

l. Pipette 100 µL of TMB Substrate Solution to all wells, including the blank wells.

m. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes, preferably on a rotator set at 100 rpm. Avoid direct exposure to intense light. The point at which the substrate reaction is stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance only up to 2.0 O.D; therefore, color development within individual microwells must be monitored and the substrate reaction stopped before positive wells are no longer properly recordable.

n. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well, including the blank wells. It is important that the acid is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately or within one hour if the microwell strips are stored at 2° to 8°C in the dark.

o. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 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 ICAM-1 standards.

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.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the soluble ICAM-1 concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating ICAM-1 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 ICAM-1 concentration.
- For samples which have been diluted 1:10, the concentration read from the standard curve must be multiplied by the dilution factor (x10).

  Note: *Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low ICAM-1 levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual ICAM-1 level.*

It is suggested that each testing facility establish a control sample of known ICAM-1 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.

Typical data using the soluble ICAM-1 ELISA

Measuring wavelength: 450 nm; Reference wavelength: 620 nm

Standard	ICAM-1 Concentration (ng/mL)	O. D. (450 nm)	O. D. Mean	C. V. (%)
1	100 100	1.737 1.806	1.772	2.8
2	50 50	1.114 1.087	1.101	1.7
3	25 25	0.570 0.546	0.558	3.0
4	12.5 12.5	0.247 0.235	0.241	3.5
5	6.25 6.25	0.110 0.100	0.105	6.7
Blank	0 0	0.004 0.006	0.005	

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.
- Improper or insufficient washing at any stage of the procedure could result in 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.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays using murine monoclonal antibodies leading to false positive or false negative results. HAMA interference can be reduced by adding murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) to the Sample Diluent.

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TEST PROTOCOL SUMMARY

1. Wash microwell strips twice with Wash Buffer.
2. Add 100 µL Sample Diluent, in duplicate, to all standard wells except those for the highest concentration (A1 and A2).
3. Pipette 100 µL soluble ICAM-1 Standard into the first standard wells and create standard dilutions ranging from 100 to 6.25 ng/mL by transferring 100 µL from well to well; Discard 100 µL from the last wells. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
4. Add 90 µL Sample Diluent, in duplicate, to sample wells.
5. Add 10 µL Sample, in duplicate, to designated wells.
6. Prepare HRP-Conjugate
7. Add 50 µL diluted HRP-Conjugate to all wells
8. Cover microwell strips and incubate 1 hour at room temperature (18º to 25ºC).
9. Empty and wash microwell strips 3 times with Wash Buffer.
10. Add 100 µL of TMB Substrate Solution to all wells including blank wells.
11. Incubate the microwell strips for about 10 minutes at room temperature (18º to 25º).
12. Add 100 µL Stop Solution to all wells including blank wells.
13. Blank microwell reader and measure color intensity at 450 nm.

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