User's Manual

Monkey sICAM-1 ELISA Kit

REF **DEIA4609**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

PRODUCT INFORMATION

Intended Use

The monkey sICAM-1 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of monkey sICAM-1. **The monkey sICAM-1 ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

General Description

Intercellular Adhesion Molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family and functions as a ligand for the Lymphocyte Function-Associated Antigen-1 (LFA-1), an alpha-beta-complex that is a member of the leukocyte integrin family of cell-cell and cell-matrix receptors. This family consists of the leukocyte adhesion glycoproteins LFA-1 which mediates lymphocyte adhesion, Mac-1 which mediates granulocyte adhesion and p150,95.

ICAM-1 is a single-chain glycoprotein with a polypeptide core of 55kD that can be expressed on non-hematopoietic cells of many lineages such as 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 dendritic cells in tonsils, lymph nodes and Peyer's patches. ICAM-1 is inducible on fibroblasts and endothelial cells by inflammatory mediators such as IL-1, TNF and IFNγ within few hours and is correlated to the infiltration of lymphocytes into inflammatory lesions.

ICAM-1 seems to be the initial marker of inflammatory reactions and is expressed prior to, and to a greater extent than is HLA-DR. The role of ICAM-1 as a disease marker has been demonstrated for a number of different indications and pathological situations.

Principles of Testing

An anti-monkey sICAM-1 coating antibody is adsorbed onto microwells.

Monkey sICAM-1 present in the sample or standard binds to antibodies adsorbed to the microwells. A HRP-conjugated anti-monkey sICAM-1 antibody is added and binds to monkey sICAM-1 captured by the first antibody.

Following incubation unbound HRP-conjugated anti-monkey sICAM-1 is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of monkey sICAM-1 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 5 monkey sICAM-1 standard dilutions and monkey sICAM-1 concentration determined.

Reagents And Materials Provided

1. 1 aluminium pouch with a **Antibody Coated Microtiter Strips** with monoclonal antibody to monkey sICAM-1

2. 1 vial (100 µl) **HRP-Conjugate** anti-monkey sICAM-1 monoclonal antibody

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3.   2 vials (500 µl) monkey sICAM-1 **Standard**, 100 U/ml

4.   1 vial (12 ml) **Sample Diluent**

5.   1 vial (5 ml) **Assay Buffer Concentrate** 20× (PBS with 1% Tween 20 and 10% BSA)

6.   1 bottle (50 ml) **Wash Buffer Concentrate** 20× (PBS with 1% Tween 20)

7.   1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

8.   1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

9.   2 **Adhesive Films**

Materials Required But Not Supplied

1.   5 ml and 10 ml graduated pipettes

2.   5 µl to 1000 µl adjustable single channel micropipettes with disposable tips

3.   50 µl to 300 µl adjustable multichannel micropipette with disposable tips

4.   Multichannel micropipette reservoir

5.   Beakers, flasks, cylinders necessary for preparation of reagents

6.   Device for delivery of wash solution (multichannel wash bottle or automatic wash system)

7.   Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

8.   Glass-distilled or deionized water Statistical calculator with program to perform regression analysis

Storage

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Specimen Collection And Preparation

Cell culture supernatant and serum (baboon, rhesus macaque) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting. Pay attention to a possible **"Hook Effect"** due to high sample concentrations (see Calculation). 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 aliquoted and must be stored frozen at -20°C to avoid loss of bioactive monkey sICAM-1. If samples are to be run within 24 hours, they may be stored at 2-8°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently. Cell culture media without serum component are not suitable for monkey sICAM-1 determination with the ELISA.

Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the monkey sICAM-1 levels determined. There was no significant loss of monkey sICAM-1 immunoreactivity detected by freezing and thawing.

b. Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the monkey sICAM-1 level determined after 24, 48 and 96 h. There was no significant loss of monkey sICAM-1 immunoreactivity detected during storage under above conditions.

Reagent Preparation

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.

1. **Wash Buffer**

Pour entire contents (50 ml) of the Wash Buffer Concentrate 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. The pH of the final solution should adjust to 7.4

Transfer to a clean wash bottle and store at 2-25°C. Please note that Wash Buffer is stable for 30 days.

Wash Buffer may also be prepared as needed according to the following table:

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

2. **Assay Buffer (1×)**

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

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

3. **HRP-Conjugate**

Please note that the HRP-Conjugate should be used within 30 minutes after dilution. Make a 1:100 dilution of the concentrated HRP-Conjugate solution with Assay Buffer (1×) in a clean plastic tube as needed according to the following table:

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

4. **Monkey sICAM-1 Standard**

Standard dilutions can be prepared directly on the microwell plate (see **Assay Procedure 3**) or alternatively in tubes.

5. **External Standard Dilution**
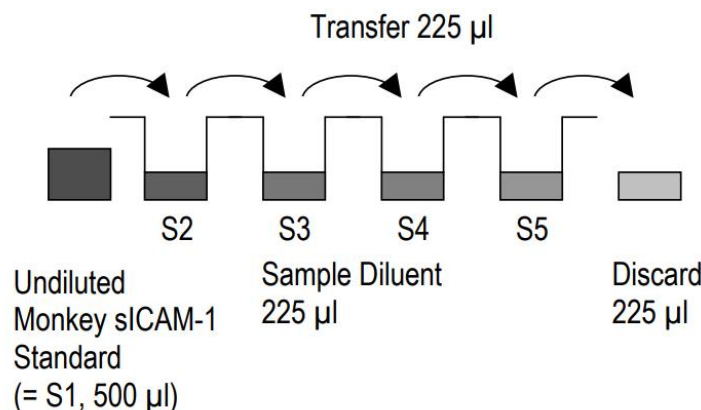
Label 4 tubes, one for each standard point.

S2, S3, S4, S5 Then prepare 1:2 serial dilutions for the standard curve as follows:

Pipette 225 µl of Sample Diluent into tubes S2-S5.

Pipette 225 µl of undiluted standard (serves as the highest standard S1, concentration of standard 1= 100 U/ml) into the first tube, labelled S2, and mix thoroughly before the next transfer (concentration of standard 2 = 50 U/ml).

Repeat serial dilutions 3 more times thus creating the points of the standard curve (see Figure).

Sample Diluent serves as blank.

Transfer 225 µl

S2   S3   S4   S5

Undiluted
Monkey sICAM-1   Sample Diluent   Discard
Standard   225 µl   225 µl
(= S1, 500 µl)

Assay Procedure

1. 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.

2. Wash the microwell strips twice with approximately 400 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10-15 seconds** before aspiration. Take care not to scratch the surface of the microwells. 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.

3. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes): Add 100 µl of Sample Diluent in duplicate to **standard wells** B1/2-E1/2, leaving A1/2 empty. Pipette 200 µl of undiluted standard (concentration = 100 U/ml) in duplicate into well A1 and A2 (see Table). Transfer 100 µl to wells B1 and B2. 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). Take care not to scratch the inner surface of the microwells. Continue this procedure 2 times, creating two rows of monkey sICAM-1 standard dilutions ranging from 100.0 to 6.3 U/ml. Discard 100 µl of the contents from the last microwells (E1, E2) used.

Transfer 100 µl

S1 S2 S3 S4 S5

Undiluted
Monkey sICAM-1
Standard
(= S1, 200 µl)

Sample Diluent
100 µl

Discard
100 µl
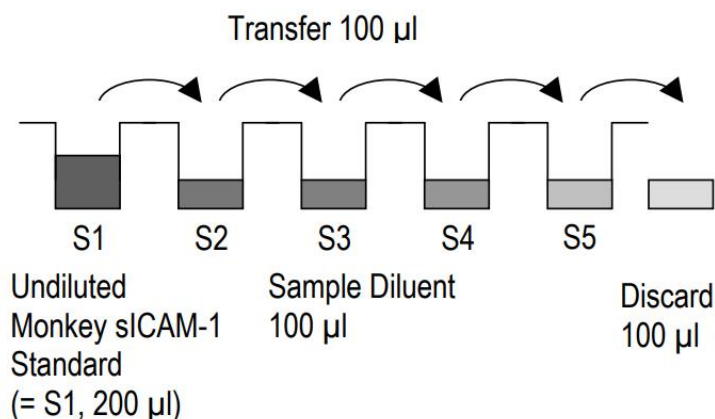
In case of an external standard dilution, pipette 100 µl of these standard dilutions (S1-S5) in the standard wells according to Table.

Table depicting an example of the arrangement of blanks, standards, control and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (100.0 U/ml)	Standard 1 (100.0 U/ml)	Sample 3	Sample 3
B	Standard 2 (50.0 U/ml)	Standard 2 (50.0 U/ml)	Sample 4	Sample 4
C	Standard 3 (25.0 U/ml)	Standard 3 (25.0 U/ml)	Sample 5	Sample 5
D	Standard 4 (12.5 U/ml)	Standard 4 (12.5 U/ml)	Sample 6	Sample 6
E	Standard 5 (6.3 U/ml)	Standard 5 (6.3 U/ml)	Sample 7	Sample 7
F	Blank	Blank	Sample 8	Sample 8
G	Sample 1	Sample 1	Sample 9	Sample 9
H	Sample 2	Sample 2	Sample 10	Sample 10

4. Add 100 µl of **Sample Diluent** in duplicate to the **blank wells**.

5. Add 90 µl of **Sample Diluent** to the **sample wells**.

6. Add 10 µl of each **sample** in duplicate to the **sample wells**.

7. Prepare **HRP-Conjugate** (see **Reagents Preparation of HRP-Conjugate 3**).

8. Add 50 µl of **HRP-Conjugate** to all wells.

9. Cover with an adhesive film and incubate at room temperature (18-25°C) for 1 hour, if available on a microplate shaker set at 100 rpm.

10. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point b. of the test protocol. Proceed immediately to the next step.

11. Pipette 100 µl of **TMB Substrate Solution** to all wells.

12. Incubate the microwell strips at room temperature (18-25°C) for about 10 min. Avoid direct exposure to intense light. **The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this Procedure) before positive wells are no longer properly recordable. Determination of the ideal time period for colour 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

colour. Alternatively the colour 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 0.60-0.65.

13. 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.

14. 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

1. Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.

2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the monkey sICAM-1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

3. To determine the concentration of circulating monkey sICAM-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 monkey sICAM-1 concentration.

4. **If instructions in this protocol have been followed samples have been diluted 1:10 (10 µl sample + 90 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (×10).**

5. **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low monkey sICAM-1 levels (Hook Effect). Such samples require further external predilution according to expected monkey sICAM-1 values with Sample Diluent in order to precisely quantitate the actual monkey sICAM-1 level.**

6. It is suggested that each testing facility establishes a control sample of known monkey sICAM1 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.

Typical Standard Curve
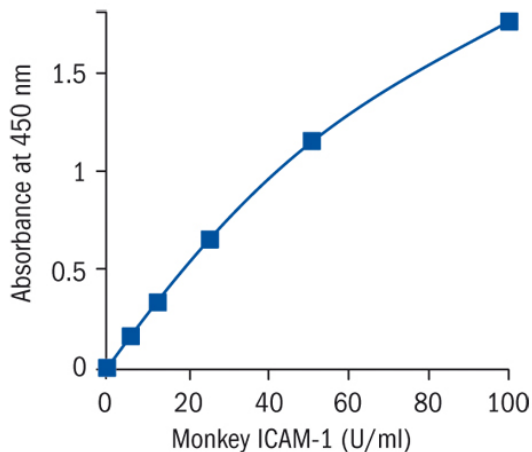
Detection Range

6.25-100 U/ml

Detection Limit

1.05 U/ml

Sensitivity

The limit of detection of monkey sICAM-1 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 1.1 U/ml (mean of 6 independent assays).

Specificity

The interference of circulating factors of the immune systeme was evaluated by spiking these proteins at physiologically relevant concentrations into a monkey sICAM-1 positive serum. There was no cross reactivity detected.

Linearity

4 serum samples with different levels of monkey sICAM-1 were analysed at serial 2 fold dilutions with 4 replicates each. The overall mean recovery was 106%.

Recovery

The spiking recovery was evaluated by spiking 4 levels of monkey sICAM-1 into Sample Diluent (serum matrix). Recoveries were determined in 3 independent experiments with 4 replicates each. The overall mean recovery was 95%.

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Reproducibility

1. **Intra-assay**

Reproducibility within the assay was evaluated in 2 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of monkey sICAM-1. 2 standard curves were run on each plate. The calculated overall intra-assay coefficient of variation was < 5%.

2. **Inter-assay**

Assay to assay reproducibility within one laboratory was evaluated in 2 independent experiments. Each assay was carried out with 6 replicates of 4 serum samples containing different concentrations of monkey sICAM-1. 2 standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was < 10%.

Precautions

1. 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.

2. Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

3. Do not mix or substitute reagents with those from other lots or other sources.

4. Do not use kit reagents beyond expiration date on label.

5. Do not expose kit reagents to strong light during storage or incubation.

6. Do not pipette by mouth.

7. Do not eat or smoke in areas where kit reagents or samples are handled.

8. Avoid contact of skin or mucous membranes with kit reagents or specimens.

9. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens. Avoid contact of substrate solution with oxidizing agents and metal.

10. Avoid splashing or generation of aerosols.

11. 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.

12. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

13. Exposure to acid inactivates the conjugate.

14. Glass-distilled water or deionized water must be used for reagent preparation. Substrate solution must be at room temperature prior to use.

15. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

16. 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.

Limitations

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

2. Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

3. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

4. 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.