DRG® Human Soluble ICAM-1 (EIA-1194)

Revised 23 Aug. 2010 RM (Vers. 2.1) **Research Use Only**

Intended Use
ICAM-1 ELISA is an *in vitro* enzyme-linked immunosorbent assay for measurement of human sICAM-1, an intercellular adhesion molecule, in cell culture supernates, plasma (EDTA and heparin), and serum. In the United States, this kit is intended for Research Use Only.
Important Note: The five vial standards have been replaced with one vial. This vial will be used to produce serial dilutions of the standard curve.

Stability and Storage
May be stored frozen at -20°C or at 2-8°C.
Allow all kit components to come to room temperature for at least 30 minutes prior to opening.

Description	Size/Vol	Form	Component usage
Anti-Human sICAM-1 Precoated 96-Well Strip Plate	1 plate	A 96-well strip plate coated with antibody specific to human sICAM-1	• Unused wells must be kept desiccated at -20°C or 2-8°C in the sealed foil pouch. • Use wells in the frame provided. • Once reagents have been added to the plate, take care NOT to let the plate DRY at any time during the assay.
Human sICAM-1 Standards	2 vials	Lyophilized. Vial contains 40 ng/ml of recombinant human sICAM-1.	• To reconstitute add 820 µl of distilled, deionized water. • Mix thoroughly by gently inverting the vial. • NOTE: Prepare just before use and use within one hour of reconstitution. • NOTE: Do not store reconstituted standards.
sICAM-1 Conjugate Reagent	8 ml	A solution with 0.06% Kathon	• Ready to use.
Sample Diluent	49 ml	A solution with 0.06% Kathon	• Ready to use.
OPD Substrate Tablets	6 each	o-Phenylenediamine Dihydrochlorine tablets	• Use non-metallic forceps to transfer the appropriate number of OPD Substrate Tablets to a 15 ml polypropylene tube.
OPD Substrate Diluent	30 ml	A solution with 0.06% Kathon	• NOTE: Prepare only the OPD Substrate Solution amount required for the number of strips being used, at 100 µl/well. • NOTE: Wear gloves when handling. • Use 5 ml of OPD Substrate Diluent per OPD Substrate Tablet. • Use a 15 ml plastic tube to prepare OPD Substrate Solution. • Do not use a tablet if it is broken or yellow in color. The prepared OPD

			Substrate Solution must be colorless to pale yellow. A yellow-orange color indicates substrate deterioration and the OPD Substrate Solution must be discarded.
Wash Buffer 20X	55 ml	A phosphate based buffer solution	• NOTE: If the Wash Buffer has formed a precipitate, dissolve by warming at 37°C before dilution. • Label a clean glass or plastic container "1xWash Buffer". • Dilute the 20x Wash Buffer 1:20 (v/v) using distilled, deionized water (e.g. 55 ml 20x Wash Buffer with 950 ml H_2O). Mix thoroughly. • 1xWash Buffer can be stored at 2-8°C, but must be at room temperature before use in this assay. • Don not use Wash Buffer if it becomes visibly contaminated during storage.

Human Soluble ICAM-1 ELISA kit. Procedural Notes

1. Materials needed but not supplied with kit include: deionized or distilled water, precision pipettes with disposable plastic tips for volumes between 5 µl and 1,000 µl; plastic pipettes to deliver 5-15 ml; glass or plastic container to prepare Wash Buffer; a squirt wash bottle or an automated 96-well plate washer; disposable reagent reservoirs; non-metallic forceps; a 15 ml polypropylene tube to prepare OPD Substrate Solution; a standard ELISA reader for measuring absorbance at 490 nm; a plate mixer capable of 150±10 rpm; latex gloves; concentrated sulfuric acid for Stop Solution; and graph paper or a computerized data reduction software package.
2. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
3. Do not use solutions containing sodium azide as it inactivates the HRP.
4. If using a multi-channel pipettor, always use a new disposable reagent reservoir and use new disposable pipette tips for each transfer to avoid cross-contamination.
5. Vigorous plate washing is essential.
6. **OPD is a known carcinogen**. Avoid contact of OPD Tablets and OPD Substrate Diluent with skin, mucous membranes and metal surfaces.
7. Sulfuric acid used for the Stop Solution is a very strong electrolyte. Take care to protect skin, eyes and clothing when handling.
8. Individual kit components contain preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.

SAMPLE HANDLING

- Serum, plasma (EDTA or heparin) or cell culture supernates may be tested in this ELISA at 10 µl per well.
- Store samples to be assayed within 24 hours at 2-8°C. For long term storage, aliquot and freeze at -70°C. Avoid repeated freeze-thaw cycles.

- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Gradually equilibrate samples to room temperature before beginning the assay. Do not use heated water baths to thaw or warm samples.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any questions about the integrity of a sample, make a note on the template and interpret results with caution.

SAMPLE DILUTION

- Before performing this assay, dilute samples 1:100. For example: add 10 µl of sample to 990 µl of Sample Diluent.
- Mix gently by inverting tubes.
- If the human sICAM-1 concentration of a sample diluted 1:100 possibly exceeds the highest point of the standard curve, prepare additional 10-fold (1:10) dilutions of these samples. For example: A 10-fold dilution is prepared by adding 50 µl of test sample to 450 µl of Sample Diluent. Mix thoroughly between dilutions before assaying. Prepare all sample dilutions using the Sample Diluent provided.

ASSAY PROCEDURE

- All standards and samples should be run in duplicate.
- Determine the number of strips required. Leave these strips in the plate frame. Place remaining unused strips in the provided foil pouch with desiccant. Make sure foil pouch is sealed tightly. Store reserved strips at 2-8°C. After completing the assay, retain plate frame for second partial plate. When using the second partial plate, place reserved strips securely in the plate frame.
- Bring all reagents to room temperature before use in the assay. Mix reagents thoroughly. Avoid foaming.
- Use the Data Templates provided to record the locations of the zero standard (blank or negative control), human sICAM-1 standards and test samples.

Standard Preparation:

1. Label six tubes, one for each standard curve point: 10, 5, 2.5, 1.25, 0.625, and 0 ng/ml. First prepare a 1:4 dilution for the first standard point (10 ng/ml) and then 1:2 serial dilutions for the remaining standard points.
2. Pipette 450 µl of sample diluent into the first tube and 300 µl into the remaining tubes.
3. Pipette 150 µl of the reconstituted standard into the first tube (i.e., 10 ng/ml) and mix.
4. Pipette 300 µl of this dilution into the second tube (i.e., 5 ng/ml) and mix.
5. Repeat the serial dilutions (using 300 µl) three more times to complete the standard curve points. These concentrations, 10, 5, 2.5, 1.25, 0.625 and 0 ng/ml are the standard curve points.

A. Sample Incubation:

1. Leaving the blank wells empty, add 25 µl of reconstituted standards or diluted test samples to duplicate wells.
2. Leaving the blank wells empty, add 75 µl of sICAM-1 Conjugate Reagent to each well containing standards or samples. Mix well by gently tapping the plate several times.
3. Carefully cover plate with a new adhesive plate cover. Ensure all edges and strips are sealed tightly by running your thumb over edges and down each strip.
4. Incubate on a plate mixer set at 150 ± 10 rpm for two (2) hours at room temperature, 22-25°C.
5. Carefully remove adhesive plate cover and wash plate three (3) times with 1x Wash Buffer as described in the Plate Washing section.

B. Plate Washing:

1. Gently squeeze the long sides of the plate frame before washing to ensure all strips securely remain in the frame.
2. Discard plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1x Wash Buffer, then discard plate contents. Repeat procedure two additional times for a total of three (3) washes. Blot plate onto paper towels or other absorbent material.
3. **NOTE**: For automated washing, aspirate all wells and wash three (3) times with 1x Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Stop Solution Preparation

1. Prepare 2N Sulfuric Acid by adding 5.8 ml of concentrated sulfuric acid to 80 ml of distilled, deionized water. Add additional water to a final volume of 100 ml.

D. OPD Substrate Solution

Prepare OPD Substrate Solution immediately before use.

1. Prepare the appropriate amount of OPD Substrate Solution per instruction in the table above and mix well.
2. Add 100 µl of the OPD Substrate Solution to each well.
3. Incubate unsealed plate **in the dark** for 30 minutes at room temperature, 22-25°C.
4. After 30 minutes, stop the reaction by adding 50 µl of prepared Stop Solution to each well.

NOTE: Stop Solution must be added to all wells before measuring the absorbance at 490 nm. Addition of Stop Solution causes an increase in absorbance of the OPD Substrate and a shift in the absorption spectrum.

E. Absorbance Measurement

Evaluate the plate as soon as possible after stopping the reaction. The absorbance may be measured up to 2 hours after addition of Stop Solution if wells are protected from light and stored at room temperature.

1. Measure the absorbance versus the substrate blank on an ELISA plate reader set at 490 nm.

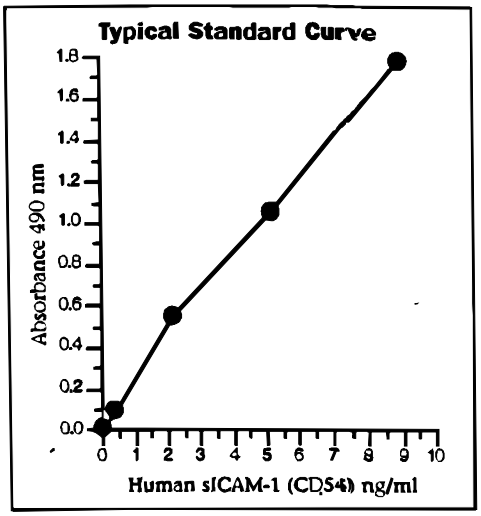
CALCULATION OF RESULTS

- The standard curve is used to determine the amount of human sICAM-1 in a sample. Generate the standard curve by plotting the average absorbance (490 nm) obtained for each standard concentration on the vertical (Y) axis vs. corresponding human sICAM-1 concentration (ng/ml) on the horizontal (X) axis.
- Calculate results using graph paper or Linear Regression Analysis data reduction software. The amount of human sICAM-1 in each sample is determined by interpolating from the absorbance value (Y axis) to human sICAM-1 concentration (X axis) using the standard curve.
- Samples have been diluted 1:100 when following the assay procedure ((10 ml sample + 990 ml Sample Diluent). Therefore, multiply the interpolated values obtained from the standard curve by 100 to calculate the ng/ml of human sICAM-1 in the sample. If additional dilutions were performed on a test sample, multiply by the total dilution factor.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Duplicate values that differ from the mean by greater than 10% should be considered suspect and repeated.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve must not be used to calculate human sICAM-1 concentrations; a standard curve must be run with every assay.

Standard Curve Example:



Interference Studies

- **Bilirubin** up to a concentration of 40 mg/dL showed no interference.
- **Lipids** up to a concentration of 12 mg/dL showed no interference.
- **Hemoglobin** at a concentration of 250 mg/dL showed no interference.