GE Healthcare

Amersham Soluble Intercellular Adhesion molecule-1 [(m)sICAM-1] Mouse, Biotrak ELISA System

Product booklet

Code: RPN2721

Page finder

1. Legal

http://www.gehealthcare.com/lifesciences

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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.

2.2. Storage

Store at 2–8°C.

2.3. Expiry

The expiry date is stated on the outer packaging and will normally be at least 4 weeks from date of despatch.

3. Description

The Biotrak™ mouse soluble intercellular adhesion molecule-1 ELISA system from GE Healthcare provides a simple, specific, reliable and precise quantitative determination of (m)sICAM-1 in cell culture supernatants, plasma and serum.

The assay system is based on a solid phase ELISA, which utilizes a monoclonal antibody for (m)sICAM-1 bound to the wells of a microplate together with an antibody to (m)sICAM-1 conjugated to horseradish peroxidase. Although GE Healthcare (m)sICAM-1 immunoassay contains recombinant (m)sICAM-1 and antibodies raised against (m)sICAM-1 it has been shown to quantitate accurately both natural (m)sICAM-1 and recombinant (m)sICAM-1.

(m)sICAM-1 can be measured in the range 25.6–1000 ng/ml (1.28–50 ng/well) in less than 4 hours using the protocol provided with the kit. Each pack contains sufficient material for 96 wells. If one standard curve is constructed, 42 unknowns can be measured in duplicate.

- High sensitivity - 5 ng/ml (0.2 5 ng/well)
- Same day protocol
- Pre-coated plate
- Specific for (m)sICAM-1

4. Introduction

Intercellular adhesion molecule-1 (ICAM-1) is a single chain glycoprotein of 90-110 kDa, which is found on the surface of the cell membrane. The protein consists of five extracellular immunoglobulin like domains, one transmembrane domain and a short cytoplasmic region. ICAM-1 is found on a large number of tissues and can be found on a range of cell types both haematopoietic and non-haematopoietic in origin. These cells include macrophages, T and B cells, fibroblasts, endothelial and epithelial cells. Normal *in vivo* expression of ICAM-1 is low, except in inflamed tissue. *In vitro* fibroblasts and endothelial cells can be activated by pro-inflammatory cytokines, such as IL-1, TNF and IFNγ. This results in a rapid increase of ICAM-1 on the cell surface. Ligands for ICAM-1 include LFA-1 and Mac-1, both being members of the β_2-integrin superfamily. The interaction of LFA-1 with ICAM-1 has been reported to demonstrate some species specificity. ICAM-1 is also the receptor for a large number of rhinoviruses.

A soluble form of ICAM-1 (sICAM-1) can be detected in the serum of normal donors. Measurement of levels of sICAM-1 may be useful in research into such diseases as multiple sclerosis, leukocyte adhesion deficiency and disseminated Hodgkin's disease; infections such as Herpes simplex or HIV; liver, gastric, colonic, gall bladder and pancreatic cancers, where matastases are present; melanoma, auto-immune diseases, bronchial asthma, rhumatoid arthritis, psoriasis, systemic lupus erythematosis and in patients with acute graft rejection.

5. Summary of the assay

This assay employs the quantitative 'sandwich' enzyme immunoassay technique. A monoclonal antibody specific for (m)sICAM-1 has been coated on the microplate provided in the kit. Samples are pipetted into the wells, and the (m)sICAM-1, if present, is bound by the immobilized antibody. After washing away any unbound sample proteins, an enzyme-linked antibody specific for (m)sICAM-1 is added to the wells and allowed to bind to any (m)sICAM-1 which was bound during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of (m)sICAM-1 bound in the initial step.

In addition to the samples to be tested, a series of wells is prepared using known concentrations of the (m)sICAM-1 standard. A curve, plotting the optical density versus the concentration of (m)sICAM-1 in these standard wells, is prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (m)sICAM-1 in the unknown samples is then determined.

6. Contents of the assay system

This pack contains the following assay components, sufficient material for 96 wells.

All reagents are stored refrigerated at 2–8°C. Refer to the expiry date on the kit box.

- **(m)sICAM-1 microplate** - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against mouse sICAM-1.
- **(m)sICAM-1 conjugate** - antibody against mouse sICAM-1, conjugated to horseradish peroxidase, 12 ml.
- **(m)sICAM**-1 standard - 2 vials of recombinant mouse sICAM-1, lyophilized.
- **Plate reagent -** with preservative, 8 ml.
- **Standard diluent -** with preservative, 12 ml.
- **Wash buffer concentrate** - 30-fold concentrated solution, with preservative, 50 ml.
- **Pre-mixed TMB substrate reagent** - with preservative, 12 ml.
- **Stop solution -** 0.18 M sulphuric acid, 15 ml.
- **Plate covers** - 4 adhesive strips.

7. Assay methodology

Users are recommended to read this entire section before starting work.

7.1 Materials and equipment required

The following materials and equipment are required:

- Pipettes or pipetting equipment with disposable tips (50 µl, 100 µl and 1.00 ml)*
- Disposable polypropylene test tubes - do not use polystyrene, polycarbonate or glass
- Measuring cylinder 2l
- Distilled or deionized water
- Plate reader capable of reading at 450 nm

Optional equipment

Assays may be performed with commercially available microplate washers to aid convenience and assay throughput.

7.2. Sample preparation

Cell culture supernatants

Centrifuge to remove any particulate material and store at -15°C to -30°C. Avoid freeze-thaw cycles.

Serum

Serum samples should be allowed to clot at room temperature. Immediately after clotting, spin down. Specimens should be clear and non-haemolyzed whenever possible. If samples contain particulate matter, clarify by centrifugation before testing.
Serum, plasma and cell culture supernatant samples that are to be assayed within 24 hours should be stored at 2–8°C. Specimens to be stored for longer periods of time should be frozen at -70°C to

avoid loss of biologically active cytokine. Avoid freezing and thawing samples more than once.

* GE Healthcare supplies a range of pipettes and disposable tips (see related products).

Dilution of test samples
Serum and plasma samples should be diluted to 1:100 in standard diluent prior to use in this ELISA. If you suspect that the (m)sICAM-1 concentration of a cell culture supernatant exceeds the highest point of the standard curve, prepare one or more five fold dilutions using the culture media. Mix thoroughly between dilutions and before assaying.

7.3. Procedural notes

1) Allow samples and all reagents to reach room temperature prior to performing the assays. Do not use water baths to thaw samples or reagents.

2) Mix samples and all reagents thoroughly before use.

3) Avoid excessive foaming of reagents. Also avoid exposure of reagents to excessive heat or light during storage and incubation.

4) Avoid handling the tops of the wells both before and after filling.

5) Standards and samples should be assayed in duplicate.

6) Run a separate standard curve for each assay.

7) The total dispensing time for each plate should not exceed 20 minutes.

8) Use only coated wells from the same reagent batch for each assay. Also do not mix reagents from different kit lots.

7.4. Assay procedure

Reagent preparation

Wash buffer concentrate

Any precipitate formed during storage will redissolve upon dilution. Dilute 30-fold to prepare 1500 ml of wash buffer. Store at 2–8°C up to the expiry date of the kit. Do not use wash buffer if it becomes visibly contaminated on storage.

(m)sICAM-1 standards

It is important that the diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured. Standard diluent will be suitable for preparation of the standard curve with serum and plasma measurements. If your samples are cell culture supernatants, the culture media will be suitable for preparation of the standard curve.

1) Reconstitute the (m)sICAM-1 standard with distilled or deionized water (reconstitution volume is stated on the standard vial label) for serum or plasma measurements. If using cell culture supernatants the cell culture media should be used to reconstitute the standard. This reconstitution produces a stock solution of 2500 ng/ml. Mix by gently inverting the vial. Use this stock solution to produce a dilution series, as described below, within the range of this assay (25.6–1000 ng/ml). Use standards within 15 minutes of dilution.

2) Label 6 tubes, one tube for each standard dilution: 1000, 400, 160, 64, 25.6 and 0 ng/ml. Then prepare 1:2.5 serial dilutions for the standard curve as follows:

3) Pipette 240 µl of appropriate diluent into each tube.

4) Pipette 160 µl of the reconstituted standard into the first tube, 1000ng/ml and mix.

5) Pipette 160 µl of this dilution into the second tube, labelled 400 ng/ml and mix.

6) Repeat the serial dilutions three more times. These concentrations, 1000 ng/ml, 400 ng/ml, 160 ng/ml, 64 ng/ml and 25.6 ng/ml together with the zero standard (0 ng/ml), which contains only appropriate diluent, are used to provide the standard curve points.

Running partial plates

This ELISA provides the flexibility to run two partial plates on separate occasions. Decide the number of strips you wish to run, leaving the strips to be used in the frame. Remove the unnecessary strips and store them in the foil pouch with the desiccant provided at 2–8°C, making sure the foil pouch is sealed tightly.

When adding the TMB substrate reagent, pour out from the bottle only the amount needed to run the first half plate. Do not combine left over substrate with that reserved for the second half of the plate. Care must be taken to ensure that the remaining TMB substrate reagent is not contaminated. **If the substrate reagent is blue prior to use, it has been contaminated. DO NOT USE.**

Assay protocol

1) Prepare assay reagents and working standards as described in the previous sections.

2) Set up the microplate with sufficient wells to enable the running of all standards and samples as required (see figure 1).

3) Remove excess microplate strips from the frame and store in the resealable foil bag.

4) Add 50 µl of the plate reagent to each well that is to be used.

5) Add 50 µl of reconstituted standard or sample per well, adding 50 µl of standard diluent or cell culture media to the zero standard wells. Cover with adhesive strip provided and incubate for 2 hours at room temperature (20–25°C).

6) Aspirate or decant each well and wash, repeating the process four times for a total of five washes. Wash vigorously by filling

each well with wash buffer (~400 µl) using a washbottle, pipette or manifold dispenser. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towelling.

7) Add 100 µl of (m)sICAM-1 conjugate. Cover with a new adhesive strip and incubate for 1 hour at room temperature (20–25°C).

8) Repeat the aspiration/wash step as in step 6.

9) Add 100 µl of TMB substrate solution into each well, incubate for 30 minutes at room temperature. If the substrate reagent is blue prior to use, do not use. **The plate should be developed in the dark**. Do not cover the plate with aluminium foil.

10) Add 100 µl of stop solution to each well.

11) Determine the optical density of each well within 30 minutes, using a spectrophotometer set to 450 nm.

Figure 1. Recommended positioning of standard (0–1000 ng/ml) and sample wells (S).

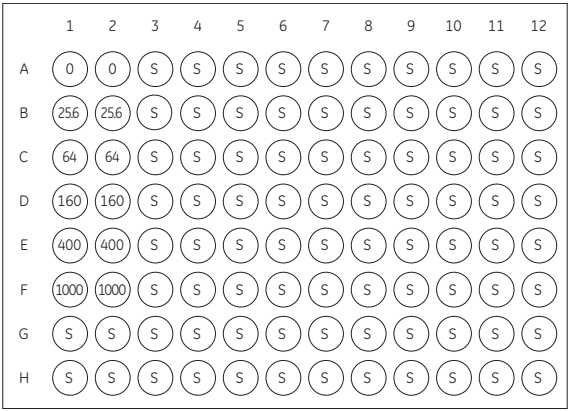


Table 1. Assay protocol (all volumes are in microlitres)

	Zero	Standards standard (B_0)	Samples
Plate reagent	50	50	50
Standard	-	50	-
Standard diluent or cell culture media	50	-	-
Sample	-	-	50
Cover plate, incubate at room temperature (20–25°C) for 2 hours.			
Aspirate/decant and wash vigorously all wells five times with ~400 µl wash buffer.			
Conjugate	100	100	100
Cover plate, incubate at room temperature (20–25°C) for 1 hour.			
Aspirate/decant and wash vigorously all wells five times with ~400 µl wash buffer.			
Substrates	100	100	100
Incubate at room temperature (20–25°C) for 30 minutes in the dark.			
Stop solution	100	100	100
Determine optical density at 450 nm within 30 minutes.			

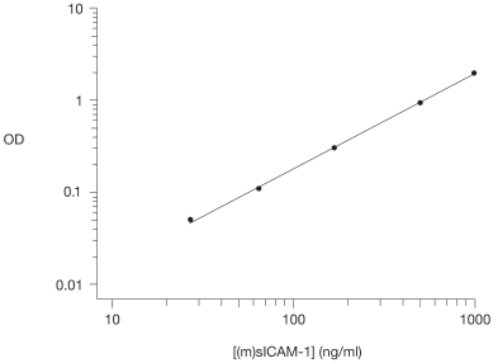
7.5. Calculation of results

Average the duplicate readings for each standard, control and sample and subtract the zero standard optical density.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using a log/log plot and regression analysis may be applied to the log transformation.

Figure 2 shows such a plot of the data from table 2. The standard curve is provided for illustration only. A standard curve should be generated for each set of samples to be assayed.

Figure 2. Standard curve



Typical assay data

The following data (table 2) were obtained for a standard curve using the protocol provided.

Table 2. Typical assay data

Tube	Optical density	Zero standard subtracted
Zero standard	0.090	-
25.6 ng/ml standard	0.138	0.048
64 ng/ml standard	0.219	0.129
160 ng/ml standard	0.428	0.338
400 ng/ml standard	0.958	0.868
1000 ng/ml standard	2.209	2.119

8. Additional information

8.1. Specificity

This assay recognizes both natural and recombinant. It does not cross react with rat sICAM-1 or human sICAM-1.

8.2. Reproducibility

Within-assay precision

The within-assay coefficient of variation of the ELISA has been determined to be <10%.

Between-assay precision

The between-assay coefficient of variation of the ELISA has been determined to be <10%.

8.3. Sensitivity

The minimum detectable dose of (m)sICAM-1 was determined to be 5 ng/ml (0.25 ng/well), by adding two standard deviations to the optical density value of zero and calculating the corresponding concentration from the standard curve.

8.4. Recovery

Natural and recombinant mouse sICAM-1 (162 ng/ml) was spiked into unstimulated and mitogen stimulated mouse splenocyte supernatants. An average recovery of 90 ± 3% was found for recombinant sICAM-1 and 98 ± 2% for natural sICAM-1.

8.5. Expected values

Levels of mouse sICAM-1 were determined in 20 normal animals by diluting samples 1:100 and 1:300. Mean levels in serum samples were 13.1 µg/ml, with a range of 10.3 to15.0 µg/ml. Mean levels in plasma samples were 11.0 µg/ml, with a range of 8.1–13.8 µg/ml.

9. References

1) ADAMS, D.H. *et al., The Lancet*, **2**, p.1122, 1989.

2) BALLANTYNE, C.M. *et al., Genomics*, **14**, p.1076, 1992.

3) FINE, J.S. and KRUISBEEK, A.M., *J. Immunol.*, **9**, p.2852, 1991.

4) HORLEY, K.J. *et al., EMBO.*, **8**, p.2889, 1989.

5) JOHNSTON, S.C. *et al., J. Immunol.,* **145**, p.1181, 1990.

6) LEEUWENBERG, J.F.M., *Immunology*, **77**(4), p.543, 1992.

10. Related products

Biotrak range of mouse cytokine ELISA systems

Interleukin-1α [(m)IL-1α]	ELISA	RPN 2719
Interleukin-1β [(m)IL-1β]	ELISA	RPN 2720
Interleukin-2 [(m)IL-2]	ELISA	RPN 2710
Interleukin-3 [(m)IL-3]	ELISA	RPN 2711
Interleukin-4 [(m)IL-4]	ELISA	RPN 2712
Interleukin-5 [(m)IL-5]	ELISA	RPN 2713
Interleukin-6 [(m)IL-6]	ELISA	RPN 2714
Interleukin-10 [(m)IL-10]	ELISA	RPN 2715
Granulocyte-macrophage colony stimulating factor [(m)GM-CSF]	ELISA	RPN 2716
Interferon-gamma [(m)IFNγ]	ELISA	RPN 2717
Tumour necrosis factor-α [(m)TNFα]	ELISA	RPN 2718

Biotrak range of human cytokine ELISA systems

Please contact your local GE Healthcare office for full details.

Range of unlabelled and radiolabelled growth factors and cytokines

Cell proliferation assay system and reagents

Cell proliferation assay system	RPN 210
Cell proliferation kit (for immunocytochemical/ immunohistochemical measurement)	RPN 20
Monoclonal anti-bromodeoxyuridine	RPN 202
Cell proliferation labelling reagent	RPN 201

Pipettes and pipette tips

Single channel, variable volume pipettes

Volume range	
0.5–10 µl	RPN 2340
5–50 µl	RPN 2341
50–200 µl	RPN 2342
200–1000 µl	RPN 2343
1–5 ml	RPN 2344

Multi-channel, variable volume pipettes

8 channel, 5–50 µl RPN 2372

8 channel, 50–250 µl RPN 2373

GE Healthcare range also includes fixed volume, 4 and 12 multi-channel variable volume pipettes, a range of pipette tips and related accessories.

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